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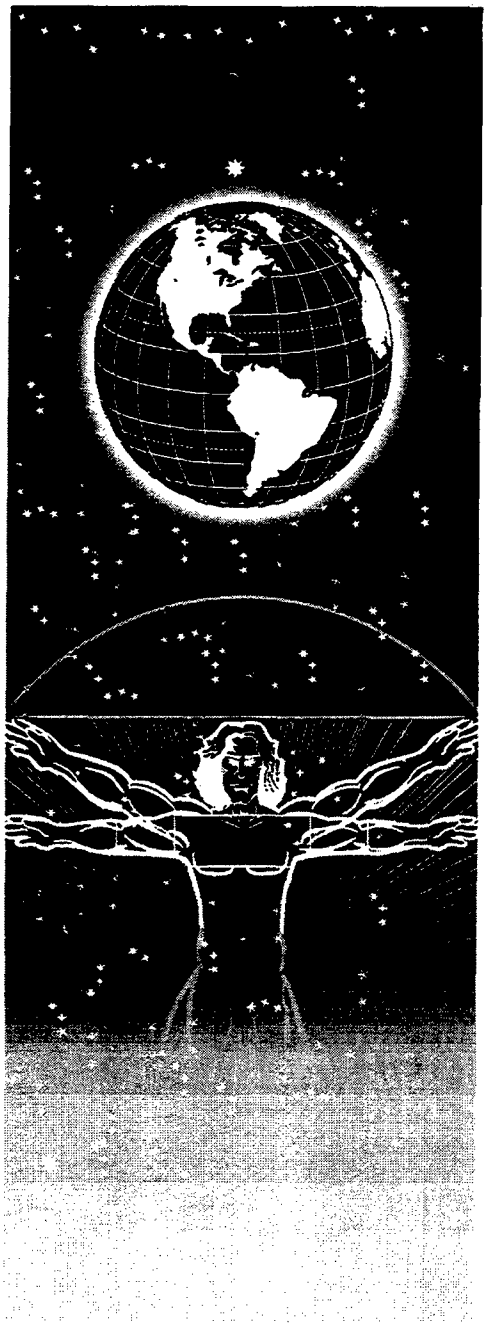

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Noncancer Effects of Trichloroethylene: Pharmacokinetics and Risk Assessment

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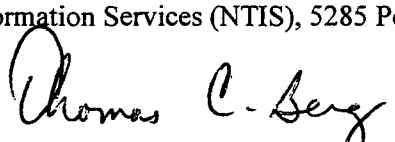

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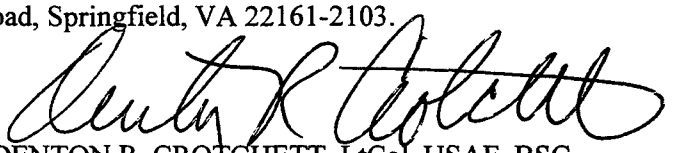

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This report provides an evaluation of the noncancer effects of trichloroethylene (TCE) exposure and presents alternatives for the development of reference doses (RfDs) and reference concentrations (RfCs). These alternatives are organized within a framework for dose-response assessment - exposure: dosimetry (pharmacokinetics): mode of action (pharmacodynamics): response. This framework provides a consistent organization within which to make scientific judgments about available information, its interpretation and use. These judgments occur in the selection of potential critical studies, choices of internal dose metrics based upon mode of action, selection of pharmacokinetic models, interspecies extrapolation pharmacodynamics, and of pharmacodynamics and selection of other uncertainty factors. Potentially limiting endpoints identified included developmental eye malformation, liver effects, immunotoxicity, and kidney toxicity form oral exposure and neurological, liver, and kidney effects by inhalation. Default analyses used the traditional no-observed-adverse-effect-level (NOAEL) divided by uncertainty factor (UF) approach, as well as the benchmark dose (BMD)/UF method. Following the default analyses, mode of action and quantitative pharmacokinetic information were incorporated. A physiologically-based pharmacokinetic (PBPK) model for TCE and it's major metabolites was used to estimate internal dose metrics for the exposure scenarios used in each experimental study. The utility of this approach was demonstrated for neurological and kidney toxicities among others. For neurological effects, use of peak trichloroethanol concentration linearized the response data, supporting its role as the active agent. The BMDs for kidney toxicity, following oral and inhalation (truncated)

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NONCANCER EFFECTS OF TRICHLOROETHYLENE: PHARMACOKINETICS AND RISK ASSESSMENT

TECHNICAL SUMMARY

This report provides an evaluation of the noncancer effects of trichloroethylene (TCE) exposure and presents alternatives for the development of reference doses (RfDs) and reference concentrations (RfCs). These alternatives are organized within a framework for dose-response assessment – exposure : dosimetry (pharmacokinetics) : mode of action (pharmacodynamics) : response. This framework provides a consistent organization within which to make scientific judgments about available information, its interpretation and use. These judgments occur in the selection of potential critical studies, choices of internal dose metrics based upon mode of action, selection of pharmacokinetic models, interspecies extrapolation of pharmacodynamics, and selection of other uncertainty factors. Potentially limiting endpoints identified included developmental eye malformations, liver effects, immunotoxicity, and kidney toxicity from oral exposure and neurological, liver, and kidney effects by inhalation. Default analyses used the traditional no-observed-adverse-effect-level (NOAEL) divided by uncertainty factor (UF) approach, as well as the benchmark dose (BMD)/UF method. Following the default analyses, mode of action and quantitative pharmacokinetic information were incorporated. A physiologically-based pharmacokinetic (PBPK) model for TCE and it's major metabolites was used to estimate internal dose metrics for the exposure scenarios used in each experimental study. The utility of this approach was demonstrated for neurological and kidney toxicities among others. For neurological effects, use of peak trichloroethanol concentration linearized the response data, supporting its role as the active agent. The BMDs for kidney toxicity, following oral and inhalation exposures, had very similar values for the kidney dose metric associated with the glutathione conjugation pathway. The human PBPK model was used to obtain human exposure doses for the internal dose metrics. Mode-of-action data from animals and humans, or default assumptions, were used for interspecies extrapolation. Data for liver and neurological effects indicated that humans are no more sensitive for these effects when the internal dose

metric was considered. The appropriate value for the uncertainty factor, a semiquantitative approach to interspecies extrapolation, was based upon this data demonstrating how this approach fits into the overall organizational framework. This analysis found that in several cases incorporation of pharmacokinetics and pharmacodynamics results in values that differ significantly from those obtained with the default methods.

INTRODUCTION

The literature regarding noncancer effects of trichloroethylene (TCE) exposure is extensive (reviewed in ATSDR 1997, Barton and Das 1996, Davidson and Beliles 1991, Gist and Burg 1995, U.S. EPA 1985). Studies of humans and experimental animals are available and each presents different challenges for the development and use of pharmacokinetic and pharmacodynamic modeling. The range of effects that have been studied is very large including biochemical, cellular, and target organ alterations. A large number of organs and organ systems have been reported to be targets at some dose in at least one study, including most prominently the nervous system, liver, and kidney. Because of the breadth of this database some of the most significant challenges for evaluating options for developing a noncancer risk assessment for TCE are the selection and interpretation of potential critical studies. Another major challenge is organization of available mode of action and pharmacokinetic data, because the selection of appropriate dose metrics for each endpoint needs to be based upon mechanistic considerations.

Many studies of exposed humans have been published including epidemiological studies of workers and the general population, controlled experimental exposures, and medical case studies of workers, overdose cases, and others. There is a substantial literature for acute effects in humans from its use as an anesthetic and from controlled human experiments to study potential neurological effects at occupational exposure limits. In contrast, efforts to determine potential chronic effects of exposure have confronted the problems that typically are associated with epidemiological studies including exposure to mixtures, difficulty demonstrating cause and effect, and limited characterization of exposure. There do not appear to be any human studies that would be considered adequate for developing Reference Doses (RfDs) and Reference

Concentrations (RfCs) (subsequently referred to as toxicity values), but human data play an important role for cross species comparisons of dose and toxic effects.

Experimental studies generally use mice and rats. Dosing regimens ranged from single doses to lifetime oral or inhalation exposures. While these studies generally involve well-characterized exposures, their use for risk assessment is critically dependent upon interpretation of the toxicological significance of the effects and interspecies extrapolations. Several factors increase the difficulty of interpreting study findings. Other than neurological and kidney toxicity, effects are rarely observed in multiple species. Few studies are done under good laboratory practice (GLP) guidelines. Few endpoints in tissues not associated with cancers have been studied in multiple experiments or by multiple laboratories. Studies report apparently contradictory results in several areas (e.g. neurotoxicity, developmental toxicity), but the exposure routes, methods, animal strains, or other factors vary, which may explain the variable results.

The relationship between the doses used for cancer and noncancer endpoints in the animal studies with TCE is worth noting. Chronic effects, particularly carcinogenicity, are often thought to occur at lower concentrations than effects arising from shorter exposures (except for developmental effects). For TCE, oral lifetime studies have all used relatively high doses: 500 to 1000 mg/kg/day for rats and 1000 - 2000 mg/kg/day for mice in oil gavage studies. Inhalation studies have covered a wider range of doses, 50 to 600 ppm. Studies of noncancer endpoints have used similar or lower doses in less-than-lifetime studies.

A consistent framework for analyzing dose-response information that reflects relevant biological processes has been evolving (Barton *et al.* 1998, U.S. EPA 1996a). Depending upon the availability of information, different methods can be used within the overall exposure-dosimetry-mode of action-response framework (Figure 1). All dose-response assessment methods begin with the identification of a toxic effect and then estimate an acceptable exposure levels protective of human health (U.S. EPA 1991, 1996a, 1996b, 1998). The default approach, in the absence of information, identifies a NOAEL (or LOAEL) and then makes assumptions about mode of action and dosimetry embodied in standard uncertainty factors and adjustments to continuous or daily

exposure (U.S. EPA 1994, Dourson 1994, Dourson and Stara 1983, Renwick 1993). An alternative to the NOAEL is the benchmark dose (BMD) method, which identifies a dose associated with a specified risk of response using statistical curve fitting to dose-response data (Crump 1984, 1995, Barnes *et al.* 1995, U.S. EPA 1995). Additional scientific information can be incorporated in dose-response analyses by using a combination of qualitative mode of action information with quantitative pharmacokinetic analysis (Barton *et al.* 1998, Clewell *et al.* 1998, Clewell and Andersen 1998). This approach has begun to be incorporated into noncancer dose-response assessments in the RfC process (U.S. EPA 1994). Use of mode of action information helps to inform the pharmacokinetic analysis (i.e., selection of an appropriate dose metric) and the extrapolations accomplished with uncertainty factors (e.g., extrapolation of less-than-chronic data or between species). Opportunities to use these approaches were evaluated here for noncancer effects of TCE. A more complete biologically based dose-response assessment would use quantitative descriptions of the mode of action (i.e. pharmacodynamics) and dosimetry (i.e. pharmacokinetics) in animals and humans. Absent relevant pharmacodynamic models, this approach is not feasible for any noncancer effects arising from exposure to TCE.

The focus of the remainder of this report will be a brief review of the toxicity database for TCE, evaluation of options for the selection of potential critical studies upon which to base dose-response values, and comparisons of the alternative methods for developing toxicity values. An abbreviated version of this report is being published as part of the U.S. EPA's reevaluation of risk assessment for TCE (Barton and Clewell, 1998).

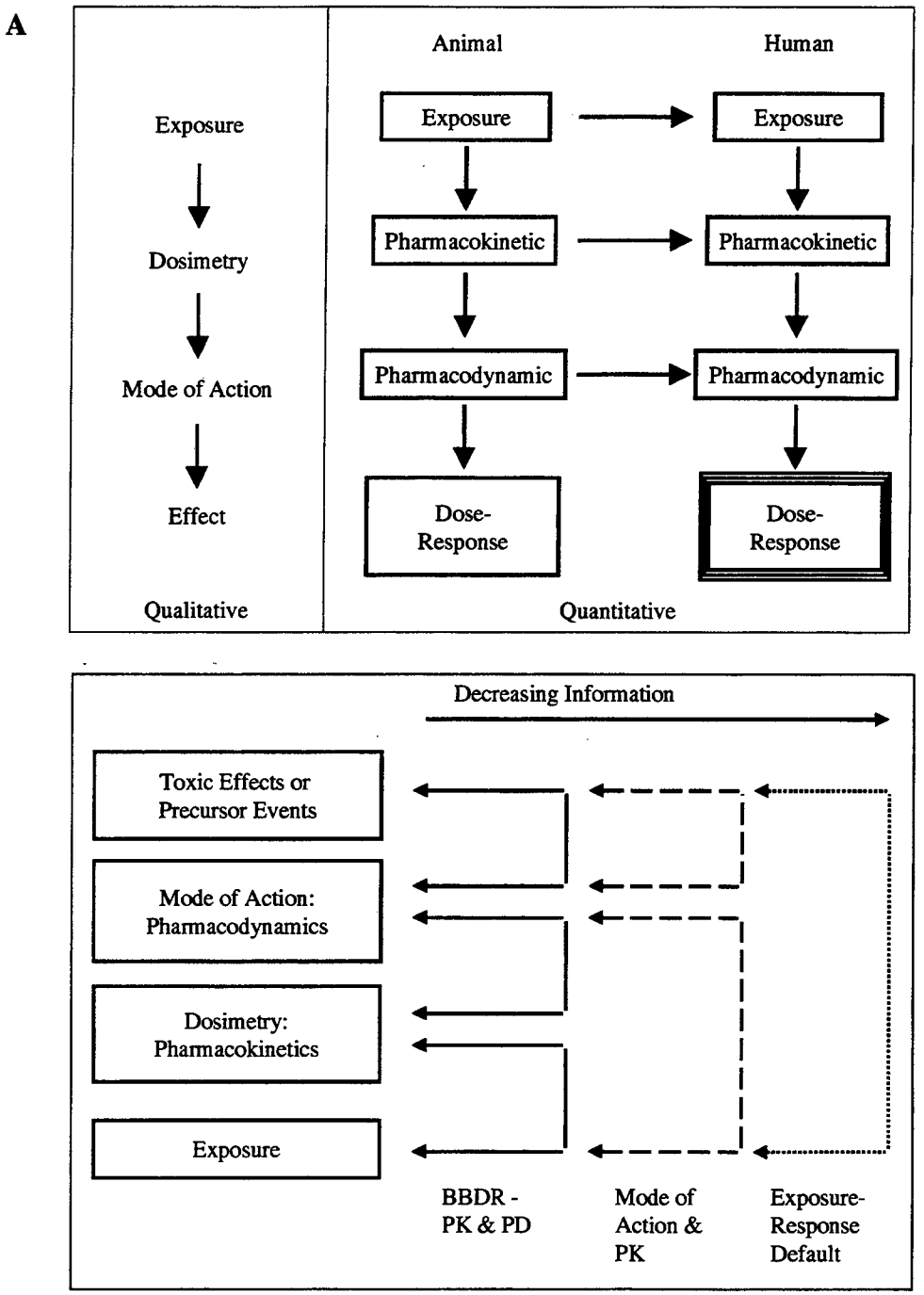

Figure 1: Framework for dose-response assessment. A) Organization of biological information used in dose-response assessment. B) Options for dose-response assessment methods. While biological processes flow from exposure to response, dose-response assessment begins with the response and works backward. The preferred method uses quantitative pharmacokinetic and pharmacodynamic models to incorporate scientific data in a biologically based dose response assessment. A more limited approach uses qualitative mode of action data to guide quantitative pharmacokinetic modeling and needed extrapolations. The low information approach relates exposure and response data using default assumptions.

IDENTIFICATION OF POTENTIAL CRITICAL STUDIES

Selection of potential critical studies is aided by well-designed studies using multiple doses. The dose-response behavior can frequently assist in the interpretation of effects observed at lower doses (U.S. EPA, 1994). Unfortunately, many studies with TCE use only one or two doses, and information on the reported effects is often unavailable at other doses. When only high dose data are reported, the studies are relatively easy to exclude from further consideration as a potential critical study because it is clear that other studies report effects at lower doses; these studies may be considered further as supporting data. When the exposure doses used are relatively low, there is no similarly easy criterion for including or excluding the study. Rather, these decisions require consideration of the strengths and weaknesses of the overall database for the effect and the scientific design and implementation of the study. The following section describes the general state of the database for endpoints from which potential critical studies might be selected with a particular focus on the potential critical studies.

Oral Studies

Oral studies have examined many potential toxic endpoints (ATSDR 1997). Endpoints discussed include neurotoxicity (and developmental neurotoxicity), immunotoxicity, reproductive toxicity, developmental malformations, kidney toxicity, and liver toxicity. A brief overview of the general knowledge in each area is provided together with discussion of specific studies that were evaluated as potential critical studies. A summary table lists the studies that were selected for further evaluation (Table 1).

Lifetime studies have predominantly focused upon cancer and used very high doses (≥500 mg/kg/day for rats and ≥1000 mg/kg/day for mice) (NCI 1976, NTP 1983, NTP 1988). These studies consistently report kidney toxicity in both species. One drinking water study lasted six months using a wider range of doses (20, 200, 400, 700 mg/kg/day) (Tucker *et al.* 1982, Sanders *et al.* 1982). This study reported small changes in gross pathology, hematology, and alterations in immunology measures.

TABLE 1: ORAL STUDIES TO BE EVALUATED QUANTITATIVELY

Effect	Study Citation	Further Quantitative	Species	Dose Route & Matrix; Doses (mg/kg/day)	Duration
Eye defects	Narotsky *et al.* 1995	NOAEL, BMD	rat	corn oil gavage 0,10, 32, 101, 320, 475, 633, 844, 1125	gestation days 6 - 15
LW/BW	Tucker *et al.* 1982	NOAEL	mouse	drinking water with emulphor 0, 18, 217, 393, 660 males 0, 18, 193, 437, 793 females	6 months
LW/BW	Buben and O'Flaherty 1985	LOAEL, BMD	mouse	corn oil gavage; 0, 100, 200, 400, 800, 1600, 2400, 3200	5 d/w, 6 weeks
LW/BW	Berman *et al.* 1995	LOAEL, BMD	rat	corn oil gavage; 0, 50, 150, 500, 1500	14 d consecutive
Immune functions	Sanders *et al.* 1982	NOAEL	mouse	drinking water with emulphor 0, 18, 217, 393, 660 males 0, 18, 193, 437, 793 females	4 and 6 months
Kidney toxicity	Maltoni *et al.* 1986	NOAEL	rat	olive oil gavage; 0, 50, 250	52 week (follow-up till natural death)

Neurotoxicity and Developmental Neurotoxicity:

TCE is known to be neurotoxic in humans and animals, particularly at high oral and inhalation doses. The human studies include medical reports from use of TCE as an inhalation anesthetic and inadvertent or intentional acute consumption of large quantities of TCE. Epidemiological studies have been performed on workers exposed by inhalation and populations drinking TCE-contaminated water (ATSDR 1997). Studies have reported changes in varied measures of neurophysiology, such as blink reflex (indicating changes in the functioning of cranial nerves) and neuropsychology (Feldman *et al.* 1988, Kilburn and Warshaw 1993). Human studies were

not considered satisfactory for developing toxicity values, although they may be considered supportive data.

Most of the neurotoxicity studies with animals use inhalation exposures, although increasingly oral data are becoming available. Several of the lifetime cancer studies reported that the rats or mice exhibited behavioral changes at 500 - 2000 mg/kg/day (e.g. NTP 1988, Henschler *et al.* 1984). Barret *et al.* (1992) report both increases and decreases in myelin thickness in rats treated with 2500 mg/kg/day for 10 weeks. At high doses transient neurotoxicity was apparent in animals; (e.g., ataxia in female rats gavaged with 633 mg/kg TCE in corn oil for 10 days) (Narotsky *et al.*1995). Oral studies using lower doses are reviewed below; all use relatively short exposures, though some are developmental.

A recent study exposed rats (corn oil gavage 0, 50, 150, 500, 1500 mg/kg/day) for up to fourteen days, followed by a functional observational battery and motor activity measurements (Moser *et al.* 1995). Activity and neuromuscular function were altered in the functional observational battery at 500 mg/kg/day. Of the three activities measured, only rearing was slightly, though significantly, increased at this dose.

Fredriksson *et al.* (1993) report three measures of spontaneous behavior among male NMRI mice (12 per dose group) dosed by gavage with emulsified TCE (50 and 290 mg/kg/day) from days 10 to 16 postnatally. Behavior was studied in three 20-minute periods at 17 and 60 days of age. No effects were seen on day 17. On day 60, mice in both dose groups spent less time rearing during the first two time periods. Differences were not seen in the final period, nor were locomotion or total activity affected during any of the three test periods.

Open field locomotor activity was evaluated in 21-day old F344 rats (F_1 generation) in a two generation reproductive study using microencapsulated TCE in the diet (NTP 1986). These animals were exposed *in utero*, by lactation, and in feed. No significant differences were identified in eight measures of behavior except for a slight decrease in the traverse time when results for male and female offspring of mothers exposed to the highest dose (approximately 300

mg/kg/d) were combined (but not separately). The second highest dose group was approximately 130 mg/kg/day for the mother.

Three papers have been published by the laboratory of D.H. Taylor looking at changes in behavior and brain histology. Taylor *et al.* (1985) evaluated exploratory activity and wheel-running among 28, 60, and 90 day old male offspring of females exposed to TCE in drinking water (312, 625, and 1250 mg/L) beginning 14 days prior to pregnancy, during pregnancy, and during lactation until pups were weaned at 21 days of age. The number of animals used for these studies is not clearly reported. No changes in exploratory behavior were seen at 28 days, but statistically significant increases were observed at 60 and 90 days. No treatment effect was seen at any time for the number of infrared beams crossed in the apparatus or for levels or timing of feeding or drinking activities. The highest dose group was more active on a running wheel around 60 days of age than were other groups. Overall, this study only provides limited evidence of effects that may be treatment related.

Isaacson and Taylor (1989) report decreases in myelinated fibers in the hippocampus of male offspring of mothers exposed during pregnancy and lactation via drinking water. Photographs of histology sections were overlaid with grids and squares without fibers stained for myelin were counted for 2 to 3 animals per treatment group. No dose-response was evident, though the authors report the decrease was statistically significant and represented a 40% decrease in the number of myelinated fibers. In addition to the small number of animals used, the information on reported dosing raises questions. The dams are reported to drink 27 ml/day of water containing 312 or 625 mg/liter, resulting in doses of approximately 4.0 or 8.1 mg/day for 56 days. The authors state that they account for degradation of TCE over a 24-hour period. Calculation of the dose without accounting for degradation would give doses of 8.4 and 16.9 mg/day indicating degradation or losses of 50% of the TCE, which appears relatively high compared to other studies.

Isaacson *et al.* (1990) report a small decrease in myelin in the hippocampus and an increased performance in a spatial navigational task following exposure to TCE in drinking water. The

exposures began at age 21 days and lasted for 4 - 6 weeks during which time Sprague-Dawley rats would be expected to grow from 50 g to 250 g or more. Limited information on water consumption provided in the paper would give an estimated average dose of about 50 mg/kg/day.

In light of effects of TCE on the nervous system at high doses, the question of whether developing animals are at greater risk is an important one. The available data, while suggestive, are not very strong and present mixed results for behavioral effects. The limited number of animals used and the lack of dose-response relationships raise concerns about the use of the findings of reduced myelination. In addition, there appear to be questions about estimating the doses. Reductions in myelination can be a matter of great concern and an important mechanism for neurological disease states in animals and humans. Therefore, this area needs to be addressed by further research. The best of the oral neurobehavioral toxicity studies used adult rats and only 14 days exposure (Moser *et al.* 1995). It reported a NOAEL of 150 mg/kg/day and a LOAEL of 500 mg/kg/day for increased rearing. These studies were considered supporting evidence, but will not be analyzed quantitatively because of other effects reported at lower doses, the study limitations discussed, and the availability of other developmental or chronic studies.

Immunological

Limited data from human and animal studies are available on immunological effects of TCE (ATSDR 1997). A recent study exposed rats and mice to TCE for three days by intraperitoneal injection (mice: 1315 mg/kg/day; rats: 6.6, 66, 660 mg/kg/day, Wright *et al.* 1991). Reduced spleen cell number or fractional spleen weight was reported at the highest doses only. Another study used a strain of mice prone to development of autoimmune disease (Khan *et al.* 1995). This study found increased autoimmune antibodies (e.g. anti-nuclear antibodies) and increased spleen weights in this mouse strain following i.p. injection with 1315 mg/kg/day.

In the only oral study, outbred CD-1 mice were exposed to TCE in drinking water with 1% emulphor (approximately 20, 200, 400, and 700 mg/kg/day) (Sanders *et al.* 1982). Ten different measures of humoral and cell-mediated immune function were reported. Exposures of males and females lasted 4 and 6 months. Overall, effects were more frequently reported in females

suggesting they were more sensitive (male mice of other strains metabolize more TCE than the females, indicating the difference is not likely to be pharmacokinetic). Often effects observed at either 4 or 6 months were not apparent at the other time point for reasons that are unclear, but might include natural age-related changes. Sometimes the difference between the naive animals (distilled water) and the vehicle control (1% emulphor) were as great as changes with TCE treatment raising further interpretive questions. The positive findings for the antibody-forming (or plaque forming) cells and the delayed hypersensitivity assay are important in light of recent analyses showing the antibody forming assay alone and the two assays together are highly predictive of immunotoxicity (Luster *et al.* 1992a, b). The authors concluded that, in females, measures of antibody-dependent immune function were affected at the two highest doses, while measures of cell-mediated immunity were affected at all four doses, but that males were relatively unaffected. ATSDR (1997) reported a NOAEL of 200 mg/kg/day and a LOAEL of 400 mg/kg/day. A single study from the same laboratory also evaluated immunotoxicity of chloral hydrate [oral gavage, 3 months, doses equivalent to 1/10 and 1/100 of the LD_{50} or 0, 0.07, 0.7 mg/kg/day (Kauffmann *et al.* 1982)]. Again male mice were unaffected, while there was a decrease in one of two measures of humoral immune function in females. This study supports the findings of effects with TCE and indicates that the immune effects are probably due to metabolites of TCE. The Sanders *et al.* (1982) study was evaluated further as a potential critical study.

Reproductive Effects

No adverse reproductive effects have been reported for humans exposed orally to TCE (ATSDR 1997). Several animal reproductive studies have been reported in the literature including two generation studies with mice and rats using microencapsulated TCE in feed (NTP 1985, NTP 1986, Cosby and Dukelow 1992, Dawson *et al.* 1993, Zenick *et al.* 1984, Manson *et al.* 1984). These studies have generally been negative for a wide range of reproductive endpoints except at very high doses (approximately 1000 mg/kg/day). In some cases, there are inconsistent results (e.g. for sperm malformation), though these may reflect differences in dosing, species, or strains of animals. Narotsky and Kavlock (1995) report delayed parturition and increased full-litter resorption following dosing with TCE by corn oil gavage (1125 and 1500 mg/kg/day). These

doses cause substantial, though transient, neurotoxicity in the mothers. Narotsky *et al.* (1995) report that parturitions were slightly delayed, but within normal limits for all animals. They also report an increase in whole litter resorptions at doses of 457 mg/kg/day and higher. This effect may be related to the slight decreasing trend for litters per pair in the NTP (1986) study with rats. The doses in the NTP (1986) study can only roughly be estimated from the information provided - approximately 40, 130, and 312 mg/kg/day from diet. Studies with trichloroacetate report increases in totally resorbed litters and other endpoints (some not observed with TCE) with water gavage doses of 300 mg/kg/day and higher (Smith *et al.* 1989). Because of the limited positive findings, difficulties in dose estimation, and the availability at similar or lower doses of better-documented effects, none of these studies were addressed further.

Developmental Malformations

As with other endpoints, there have been limited findings of developmental effects from TCE in human studies (some are discussed below) but they are not adequate for quantitative analysis (ATSDR 1997). There are also limited animal studies focused upon specific endpoints for developmental malformations using oral dosing. Inhalation exposures have been reported to produce no gross malformations (Hardin *et al.* 1981 - 500 ppm; Schwetz *et al.* 1975 - 300 ppm), but limited changes such as apparent delays in ossification of bones have been reported (Dorfmueller *et al.* 1979 -1800 ppm). The two effects reported following oral exposures are eye and cardiac malformations, neither of which was reported in the inhalation studies, although there are some questions about the ability of the methods and the number of pups in those studies to detect these malformations.

Eye malformations were reported following *in utero* exposures (gestation days 6 - 15) of Sprague-Dawley rats to 1125 and 1500 mg/kg/day (Narotsky and Kavlock 1995). No other malformations were reported though it is not clear how extensively others were looked for. The eye malformations are described as a reduction in the ocular bulge (microphthalmia) or its absence (anophthalmia). A second study again reported eye malformations (Narotsky *et al.* 1995). Doses ranged from 10 - 1125 mg/kg/day but no effects were apparent below 101 mg/kg/day (8 - 10 litters per dose group). The response at the highest dose in two separate

experiments was 8.7% (+ 4.8% SE) and 30%(+11% SE) of total pups affected or 38% (3 of 8) and 100% (5 of 5) of litters affected. This indicates that there is much greater variability between experiments than might be expected based upon the response data available with single experiments at multiple doses. A positive dose-response was observed in the study of Narotsky *et al.* (1995) and was analyzed quantitatively.

Studies with oral gavage TCA or DCA exposures of Long-Evans rats also report eye malformations including microphthalmia, anophthalmia, and skeletal decreases in orbit size (Smith *et al.* 1989, 1992). There was a very shallow dose-response for eye malformations (0 - 2.4% of pups affected or 0 - 14% of litters affected) with a wide range of DCA exposures (14 to 2400 mg/kg/day). A steeper dose-response was seen with TCA for which no effects were observed at 330 mg/kg/day. At 800, 1200, and 1800 mg/kg/day, affected pups increased from 3 to 17% and affected litters from 18 to 38%.

Cardiac malformations have been reported in a study using TCE or dichloroethylene in drinking water provided females prior to pregnancy, prior to and during pregnancy, and only during pregnancy (Dawson *et al.* 1993). Cardiac malformations were of interest because an increase was reported among children whose mothers were exposed to drinking water containing 1,1-dichloroethylene and TCE (Goldberg *et al.* 1990). Concentrations of TCE were measured in 1981 and ranged between 6 and 239 ppb while dichloroethylene levels were between 5 and 10% of the TCE levels. One limitation of this study is that it was not determined if people in the potentially exposed population had, in fact, consumed contaminated water. In a study of 621 nurse-anesthetists who worked during pregnancy there was no increase in cardiac malformations in their children compared to controls, although there was a significant increase in birth defects (Corbett *et al.* 1974). No information is provided on the anesthetics used, but the same authors documented levels of TCE in a different hospital (Corbett *et al.* 1973).

In an earlier study, cardiac malformations had been observed when neat TCE was placed directly in the uterus of pregnant rats (Dawson *et al.* 1990). A study reporting cardiac malformations in chicks exposed in the egg may also provide supporting evidence for this effect (Loeber *et al.*

1988). It should be noted that another study with chick eggs found TCE to cause numerous malformations in chicks (Bross *et al.* 1983) which is not the case for rodents exposed to TCE (Healy *et al.* 1982, Schwetz *et al.* 1975, Dorfmueller *et al.* 1979, Dawson *et al.* 1993).

Trichloroacetate dosed orally (330 - 1800 mg/kg/day) has been reported to produce cardiac malformations in rats (Smith *et al.*, 1989, P. D. Johnson, personal communications). More detailed studies have been carried out with DCA (14, 140 - 2400 mg/kg/day) (Epstein *et al.* 1992, Smith *et al.* 1992). Dichloroacetate was maternally toxic at all doses tested except the lowest and a positive dose-response for cardiac malformations was observed. Further studies showed the window of susceptibility to be days 9 - 11 (Epstein *et al.* 1992). The methods used in the studies with the chloroacids are not the same as those used in studying TCE, so it is unclear if they would both identify the same fetuses as affected.

The studies from the University of Arizona (Dawson *et al.* 1990; Dawson *et al.* 1993; Loeber *et al.* 1988) determined cardiac malformations using dissection of the pup hearts under a microscope. The drinking water study used TCE concentrations of 1.5 ppm and 730 (incorrectly reported in the paper as 1100) ppm from which the amount of TCE consumed was calculated based upon average water consumption during a week (Dawson *et al.* 1990). A small nonstatistically significant increase in malformations was reported at both doses when exposure was prior to pregnancy only. Exposure prior to and during pregnancy produced an increase with dose (statistically significant at the high dose); exposure only during pregnancy resulted in similar statistically significant increases at both dose levels. Exposures at 250 ppb have been completed and preliminary results appear to indicate that no effect was seen at this level, which was selected to be similar to the highest contamination level reported for the Tucson drinking water (P.D. Johnson, personal communication). It should also be noted that dichloroethylene was about 10-fold more potent than TCE in this study.

Interpretation of the Dawson *et al.* (1993) study is difficult. Determining if the effect can be reproduced in another laboratory is very important for evaluating the significance of these results. Several hypotheses about the results might be considered. The first hypothesis is that the effect

is due to TCE or its metabolites, perhaps TCA. The second hypothesis is that the exposures are responsible for the effect, but not the chemical (e.g. the opposite of a placebo effect).

Evidence to support one or the other of these hypotheses depends upon the technical design and implementation of the study and interpretation of the results. To its credit the study uses large numbers of animals and the hearts were analyzed blind (i.e. investigators did not know from which group the heart came until after dissection). The doses did not appear to cause maternal toxicity or decreased reproductive success (i.e. live births, implants, and resorptions). A difficulty with the data analysis is the lack of analysis by litter, but visual examination of the data suggest there was no litter effect (P. D. Johnson, personal communication). The use of oral and intrauterine dosing routes (drinking water and direct uterine exposure), may suggest that the effect is chemical related. Alternatively, it may suggest that both routes "stressed" the dams leading to increases in relatively subtle developmental alterations. Similar responses were obtained regardless of route.

The inconsistent dependence of increased incidences on dose is difficult to interpret. The incidence of malformations increases about three-fold with dose (controls 3%, low dose 5.5%, high dose 10.4%) among animals exposed during pregnancy only, while the dose of TCE increased approximately 450-fold (low dose 0.18 mg/kg/day; high dose 84 mg/kg/day). The higher dose is in a range where metabolism increases with exposure dose, so there would be expected to be a similarly large increase in TCA exposure of the fetuses during cardiac development if that were the active species. That such a large increase in dose produces such a slight increase in response raises doubts whether the chemical is the active agent.

Unfortunately, the available data for cardiac malformations and TCE kinetics were obtained in three strains of rats complicating the analysis (F344, Sprague-Dawley, and Long Evans). Trichloroacetate pharmacokinetics in non-pregnant female F344 and male Sprague-Dawley rats (Fisher 1987, Fisher *et al.* 1989, Larson and Bull 1992a,b) appear fairly similar so it was assumed that rat strain did not have a major impact on the data. The modeled AUCTCAs for TCE exposures of 0.2 and 84 mg/kg/day during pregnancy are 2 and 313 mg-h/L, respectively.

By comparison, the AUCTCAs estimated for TCA dosing at 330 mg/kg/day is 5396 mg-h/L using data from Fisher (1987) or Larson and Bull (1992a,b). This TCA dose produced about a 5% incidence of cardiac malformations among pups as compared to a 5 and 10% incidence with TCE dosing. There is a significant discrepancy that must be explained based upon differences in methods, strains of rats, or choice of dose metric. If TCA is the active species and the other factors are not significant, it would suggest that area under the concentration curve in the maternal blood is not a good dose metric; a dose metric in fetal tissue appears to be required.

The dependence on timing of exposure is also very difficult to interpret. The exposure prior to pregnancy led to a small increase in malformations although TCE and its metabolites (including TCA) would be expected to be largely cleared within 2 - 4 days (Fisher 1987, Fisher *et al.* 1989). Thus, the internal concentrations of TCE and metabolites during pregnancy would be much greater during cardiac development when exposures occurred during, rather than prior to, pregnancy. If only atrial septal defects are considered rather than total malformations, there was no dependence upon timing, although there was a small dose response. Clearance of DCA from adult animals is much faster due to its metabolism, and far less DCA is produced from TCE than is TCA. Thus all chemicals would be expected to be absent by the time cardiac tissue formation began around gestation day 9 and continuing through day 13 (Lau and Kavlock, 1994). Thus, it is not clear that these data indicate an effect of TCE or its metabolites.

Exposure both prior to and during pregnancy increased the incidence of malformations equally in both dose groups. (There is a mistake in the publication describing the dose for this low dose group that was confirmed with one of the authors, P.D. Johnson. The average total TCE consumed was 3.84 µl, not 23.5 µl, based upon an average daily dose of 0.04 µl/day.) These data again provide little support for either hypothesis in light of the lack of dose-response and a lack of clear effect of the timing of exposure.

This study will not be used for further quantitative analysis due to the issues raised here. However, options for addressing this and other "limited positive" data will be addressed further in the discussion of uncertainty factors. From the perspective of noncancer risk assessment for

TCE, further research on this effect is highly desirable. Experiments need to be designed to compare the two causative hypotheses - chemical vs. treatment. A research program could include: a more complete dose-response, efforts to demonstrate the role of chemical versus treatment, and elucidation of the mechanism.

Kidney Toxicity

Several studies of humans exposed to TCE either in drinking water or through accidental ingestion provide no evidence for kidney disease (ATSDR 1997). A study reporting increased urinary tract infections in children included no direct measures of kidney function (Lagakos *et al.* 1986). Workers exposed to TCE were reported to generally show no significant difference in urinary proteins, though one subgrouping of workers by age was significantly different (Nagaya *et al.* 1989).

Kidney toxicity in male and female rats (500 and 1000 mg/kg/day) and mice (1000 and 2000 mg/kg/day) is strongly associated with chronic corn oil gavage exposure to TCE (NCI 1976, NTP 1983, NTP 1988, Maltoni *et al.* 1986, Maltoni *et al.* 1988). Subsequent to observing effects at the end of the 2-year study, a 90-day study in F344 rats was reevaluated and very mild indications of toxic nephrosis were observed. Although there are reports of increased kidney weight in short exposures, it is unclear if this is related to or represents a reliable indicator of chronic toxicity (Berman *et al.* 1995, Stott *et al.* 1982). A chronic study in rats reporting kidney toxicity is evaluated quantitatively (Maltoni *et al.* 1986).

Liver Toxicity

Very limited data are available regarding liver toxicity in humans from oral exposures (ATSDR 1997). Case studies of ingestion include one that reports liver damage and several that do not.

Liver effects in animals are the best-characterized noncancer endpoint associated with tce (barton and das, 1996). Numerous measures of effects have been reported including alterations in liver to body weight ratio (LW/BW), largely due to hypertrophy and some hyperplasia (elcombe *et al.* 1985, stott *et al.* 1982), peroxisome proliferation, altered serum levels of liver enzymes (e.g. SGPT), and histopathologically observable changes including necrosis (table 2).

TABLE 2: ORAL STUDIES REPORTING LIVER EFFECTS

References	Species	Endpoints	Dose route & matrix; doses (mg/kg/d)	Duration & frequency
Borzelleca *et al.*, 1990	Rats	LW/BW ratio	aqueous emulsion (5% emulphor) 0, 100, 250, 400	1 d
Buben and O'Flaherty, 1985	Mice	LW/BW ratio, serum enzyme levels	corn oil gavage 0, 100, 200, 400, 800, 1600, 2400, 3200	6 wk, 5 d/wk
Elcombe *et al.*, 1985	Mice	LW/BW ratio DNA/cell histopathology	corn oil gavage 0, 500, 1000, 1500	10 d consecutively
Elcombe 1985	Mice	LW/BW ratio, palmitoyl CoA oxidation	corn oil gavage 0, 50, 100, 200, 500, 1000, 2000	10 d consecutively
Goel *et al.*1992	Mice	LW/BW ratio, δ-aminolevulinic acid dehydratase, histopathology	groundnut oil gavage 0, 500, 1000, 2000	4 wk, 5 d/wk
Melnick *et al.*, 1987	Rats	LW/BW ratio	microencapsulated in diet 0, 600, 1300, 2200, 4800	14 d consecutively
Merrick *et al.*, 1989	Mice	LW/BW ratio	corn oil gavage or aqueous emulsion 0, 600, 1200, 2400 (males) 0, 450, 900, 1800 (females)	4 wk, 5 d/wk
Stott *et al.*, 1982	Mice	LW/BW ratio	corn oil gavage 0, 250, 500, 1200, 2400	3 wk, 5 d/wk
Tucker *et al.*, 1982	Mice	LW/BW ratio	drinking water 0, 18, 217, 393, 660 (males) 0, 18, 193, 437, 793 (females)	6 mths

Some of these effects (e.g. LW/BW, peroxisome proliferation) are not typically considered adverse effects in themselves, while others (e.g. SGPT or histopathology) are. Effects in rats were less apparent than in mice (Stott *et al.* 1982, Elcombe *et al.* 1985). No histopathological effects associated with TCE were reported in chronically exposed rats (NTP 1988, NCI 1976). This is notable because the doses used (500 and 1000 mg/kg/day) cause increased LW/BW in shorter exposures (e.g. Berman *et al.* 1995). Data for noncancerous liver changes in mice exposed for a lifetime are not available because the studies only report liver cancers (NCI 1976, NTP 1983). The six-month drinking water study in mice reported that gross pathology was "unremarkable", though fatty infiltration was observed in 11 of 59 animals from all treatment groups that were killed at six months (Tucker *et al.* 1982). Increased LW/BW was present in high dose females (800 mg/kg/day) and males of the three higher dose groups (217, 393, and 660 mg/kg/day).

The ratio of liver to body weight is a reasonable candidate as an early indicator for subsequent toxicity. However, there are significant issues about the effects of corn oil, the causal relationship to liver toxicity, the role of peroxisomal proliferation, and interspecies comparisons. Increases in LW/BW are reported at doses as low as 50 mg/kg/day (Berman *et al.* 1995) and 100 mg/kg/day with corn oil gavage (Buben and O'Flaherty 1985), and 217 mg/kg/day with drinking water (Tucker *et al.* 1982) (see Table 2).

Increased LW/BW was reported after exposures to TCE by corn oil gavage and aqueous emulsion with emulphor (450 to 1800 mg/kg/day, Merrick *et al.* 1989). Leakage of lactate dehydrogenase increased at 600 mg/kg/day with TCE in corn oil, but not aqueous gavage. No effect of vehicle was apparent for two other serum enzymes measured.

Data on increases in palmitoyl-coenzyme A (CoA) oxidase activity (Elcombe 1985), a marker of peroxisome proliferation, show a similar dose-response to that reported by Buben and O'Flaherty (1985) although the strains and dosing regimens used are not identical. Other reported changes included decreased DNA/cell, indicating hypertrophy, and increased incorporation of labeled nucleotides, suggesting cell proliferation, at 500 mg/kg/day and higher (Elcombe *et al.* 1985).

Increases in LW/BW in mice are greater than in rats, which parallels the greater increase in peroxisome proliferation in the mice (Elcombe *et al.* 1985). These and other data may indicate that the increased LW/BW is at least partly due to peroxisomal proliferation.

Alterations in histopathology or liver enzymes have been reported at 500 mg/kg/day in mice (Elcombe *et al.* 1985, Goel *et al.* 1992, Stott *et al.* 1982). Small, statistically nonsignificant, decreases in liver glucose-6-phosphatase (G6P) activity were reported at 100 mg/kg/day and higher, degeneration at 400 mg/kg/day, and statistically significant increases in G6P at 800 mg/kg/day (Buben and O'Flaherty 1985). Increased lactate dehydrogenase occurred at 600 mg/kg/day in corn oil and 1200 mg/kg/day in aqueous emulsion (Merrick *et al.* 1989). Berman *et al.* (1995) report histological effects at 1500 mg/kg/day and greater in rats. The altered oxidative environment occurring with peroxisome proliferation in mice and rats may also play a role in the liver damage indicated at high doses by measures such as leakage of serum enzyme levels.

Good data describing the dose-response relationships for increased LW/BW are available from several of these studies. Unfortunately, the drinking water study, which might be considered the most relevant exposure and which has the longest duration, does not report the actual values for LW/BW, but it was considered further because the NOAEL dose was identified (Tucker *et al.* 1982). Two other studies reporting altered LW/BW ratios also were evaluated further (Buben and O'Flaherty 1985, Berman *et al.* 1995).

Inhalation Studies

Most of the toxicity endpoints that have been observed in oral studies have also been observed with inhalation exposures. The exceptions are endpoints that have been studied by only a single laboratory using one exposure route. The endpoints discussed below include neurotoxicity, immunotoxicity, reproductive toxicity, developmental malformations, kidney toxicity, and liver toxicity. A brief overview of the general knowledge in each area is provided together with discussion of specific studies that were evaluated as potential critical studies. A summary table lists the studies that were selected for further evaluation (Table 3). The only available chronic

inhalation studies are those of Maltoni *et al.* (1986, 1988) which reported kidney toxicity in male rats, but not in mice or female rats.

TABLE 3: INHALATION STUDIES TO BE EVALUATED QUANTITATIVELY

Effect	Study citation	Further quantitative Evaluation	Exposure concentration (ppm)	Species	Duration
Electroencephalographic changes, heart rate	Arito *et al.* 1994	LOAEL, BMD	50, 100, 300	rat	8 h/d, 5 d/w, 6 weeks
LW/BW	Kjellstrand *et al.* 1983a	LOAEL, BMD	37, 75, 150, 300	mouse	Continuous, 30 day
Kidney toxicity	Maltoni *et al.* 1986	NOAEL, BMD	100, 300, 600	rat	7 h/d, 5 d/w, 104 weeks

Neurotoxicity and Developmental Neurotoxicity

Inhalation of TCE is well known to have neurological effects both in humans and animals, hence its use as an anesthetic (ATSDR 1997, Annau 1981). Acute and occupational studies of behavioral effects in humans variably report no effects or changes in a variety of neurological measures, e.g., after exposures at moderate concentrations of 100 ppm to 200 ppm (Annau 1981, ATSDR 1997). At higher doses effects become obvious with anesthesia resulting around 1000 ppm. Studies of workers chronically exposed to TCE report a range of neurological effects, but often the TCE levels are unquantified or only urinary TCA levels are reported. A few case studies have reported cardiac arrhythmia or other cardiac effects after unspecified or high inhalation exposures, while a brief exposure to 200 ppm for 2.5 hours had no effects (ATSDR 1997). Cardiac arrhythmias are associated with a broad range of chlorinated hydrocarbons and are due to sensitization to catecholamines.

Studies in animals have focused upon physical (e.g. biochemical, histological, or electrophysiological) or behavior changes, but rarely both. Short duration studies using fairly

high concentrations (typically $\leq$ 1000 ppm) have focused on several endpoints including effects on trigeminal nerves and hearing loss (ATSDR 1997). Animal studies using moderate concentrations and exposure durations of 5 months or less report effects at approximately the same range as reported with humans. No neurotoxicity data are available from chronic inhalation studies.

A study of heart rate and electroencephalographic responses during wake and sleep periods reports alterations at 50, 100, and 300 ppm in rats exposed for six weeks (Arito *et al.* 1994). Measurements were made during the exposure and during a 22-hour postexposure period. Statistically significant changes were observed at several doses during or post exposure for time spent in wakefulness, slow-wave sleep, and heart rate. This study was evaluated quantitatively below because it reports effects following subchronic exposure.

Several other studies report behavioral effects. Changes in exploratory behavior were observed in familiar and unfamiliar (an 'exploration-thirst' test) territories using rats exposed to 100, 200, 500, and 1000 ppm for 12.5 weeks (Silverman and Williams 1975). Exposure decreased activity in a familiar setting, while water was obtained more rapidly in the 'exploration-thirst' test. Effects became apparent earlier at higher doses. A decrease in shock avoidance was reported at 125 ppm (Goldberg *et al.* 1964). At 200 ppm increases in ambulatory, grooming, and rearing behaviors were observed (Savolainen *et al.* 1977). This appears to be inconsistent with findings of decreased activity in other studies (Silverman and Williams 1975, Arito *et al.* 1994), though it may reflect other differences such as the short exposure (5 days). Decreases in shock avoidance were also reported at 250, 500, 1000, and 2000 ppm (Kishi *et al.* 1993). Among rats exposed to 500, 1000, and 1500 ppm for 18 weeks there was a progressive change in responding in a two-choice visual discrimination task, but not in spontaneous activity, grip strength, coordinated hind limb movement, or rearing (Kulig 1987). These studies will not be analyzed further because it is unlikely that they would result in lower dose-response values than the Arito *et al.* (1994) study. While some of these studies lasted longer than the Arito *et al.* (1994), all the studies are subchronic in nature. These studies provide supporting evidence for effects following subchronic exposure at doses similar to and higher than the Arito *et al.* (1994) study.

Several studies of physical brain changes have also been reported. Changes in brain proteins in Mongolian gerbils were reported following three-month exposure to 60 or 320 ppm (Haglid *et al.* 1980, 1981). Decreases in brain specific gravity, sciatic nerve regeneration, and brain acid phosphatase activity were reported following exposure of rats, mice, or gerbils to 150 ppm for 30 days (Kjellstrand *et al.* 1982, Kjellstrand *et al.* 1987, Westergren *et al.* 1984). Savolainen *et al.* (1977) report a statistically significant decrease in RNA in rat brains following exposures of 200 ppm. Transient changes in electroretinal responses were found in rabbits exposed to 350 or 700 ppm for twelve weeks (Blain *et al.* 1994). These changes were reversed during a six-week postexposure period. This study is also of interest because they report that two measures correlated with plasma levels of trichloroethanol, while a third correlated with levels of TCE. Little or no effect was observed in peripheral nerve conduction times following exposure of rats to 500, 1000, or 1500 ppm TCE for 18 weeks (Kulig *et al.* 1987). These studies may be considered to provide supporting evidence, but will not be evaluated further. Either they do not appear to provide appropriate data for developing toxicity values or the doses used are higher than those used in the Arito *et al.* (1994) study and would likely result in a high toxicity value.

Immunotoxicity

A few studies report decreases in mortality from respiratory infections and other diseases partially indicative of human immune status, though none include biochemical or cellular measures of immune system functions (Gist and Burg 1995; ATSDR 1997). An epidemiological study of workers exposed predominantly by inhalation at Hill Air Force Base found decreased mortality from respiratory diseases including bronchitis (Spirtas *et al.* 1991).

Two studies are available in animals exposed by inhalation (Aranyi *et al.* 1986; Hobara *et al.* 1984). Hobara *et al.* (1984) report a dose-dependent decrease in leukocyte counts after one-hour exposures to 500 ppm and higher, but no statistical analysis is presented. This limited study was considered too preliminary for further analysis. Bacterial challenge of CD-1 mice following TCE exposure has been used to demonstrate compromise of pulmonary immune function (Aranyi *et al.* 1986). Following single exposures of 3-hr to 0, 2.5, 5, 10, 25, or 50 ppm, the mice were

exposed to a potentially lethal bacterial challenge. A dose-response for increased mortality was observed. A repeated 5-day exposure showed increased mortality at the 2.5 ppm level, but the increase was substantially less than predicted from the assumption that the concentration-time product would be constant. A dose-response for mortality due to bacterial challenge was also found using 50, 100, and 200 ppm (Park *et al.* 1993). Other endpoints showing dose-response increases in this study were bacterial survival in the lungs and bacterial capsulization. One factor responsible for these effects is a dose-dependent decrease in phagocytosis by lung macrophages, which would impair removal of the bacteria from the lungs (Park *et al.* 1993). In the absence of data for longer exposures and given the lack of a constant concentration-time product, it is difficult to extrapolate these acute effects for evaluating potential chronic toxicities. These data were reconsidered when evaluating the RfCs derived from other studies.

Reproductive and Developmental Effects

No reproductive studies in humans are available (ATSDR 1997). Studies in animals report effect at concentrations of 500 ppm or higher, so they will not be evaluated further because other endpoints are reported to occur at lower concentrations. Data for developmental toxicity in humans are limited (ATSDR 1997). One case control study of occupationally exposed men and women reports an increase in spontaneous abortions among women reporting TCE exposure, but the number of cases (10) is very small (Windham *et al.* 1991). A cohort study found no increase in malformations in children born to TCE exposed workers (Tola *et al.* 1980). Finally, a study of nurse-anesthetists in Michigan found an increase in birth defects among their children as compared to controls (Corbett *et al.* 1974). No information on the anesthetics being used is included, although the authors did an exposure study in a Canadian hospital, which used TCE and found up to 100 ppm (Corbett *et al.* 1973). Studies in animals report no statistically significant increases in malformations in studies using concentrations ranging from 100 - 1800 ppm (Dorfmueller *et al.* 1979, Hardin *et al.* 1981; Healy *et al.* 1982, Schwetz *et al.* 1975). One study reports an increase in delayed ossification and whole litter resorptions following exposure of rats to 100 ppm (Healy *et al.* 1982).

Kidney Toxicity

Several studies of workers have reported limited findings of renal toxicity (ATSDR 1997). Kidney toxicity was reported in an inhalation study exposing Sprague-Dawley rats to 0, 100, 300, or 600 ppm (Maltoni *et al.* 1988). Renal megalonucleocytosis was observed in male rats only in a dose-related trend. This finding is in contrast to the oral gavage data that showed effects in both sexes of rats and in mice (NTP 1988, NTP 1983). In a study of rats exposed to 35 or 700 ppm for up to 90 days, no histopathological changes were observed. This study was considered supporting evidence of toxicity at concentrations greater than 100 ppm (the NOAEL), but will not be evaluated further because other studies report effects at lower doses.

Lung Toxicity

There is very little human data reporting pulmonary toxicity (ATSDR 1997). A study in rats exposed to for up to 90 days to 700 ppm TCE reported no histopathological changes (Prendergast *et al.* 1967). A single inhalation study reports lesions in lungs of female mice exposed at concentrations of 20 to 2000 ppm (Odum *et al.* 1992). The lesions are vacuolation of Clara cells that reportedly increase in a dose-related trend, though no quantitative data are presented. A measure of cytochrome P450 activity in Clara cells isolated from these animals decreases with dose. This effect is not observed in rats and is believed to be specific to mice. It appears related to accumulation of chloral in mouse Clara cells that may be related to low metabolism to trichloroethanol or to a uniquely low level of trichloroethanol glucuronidation capacity in mouse tissue. This study will not be analyzed further due to the limited documentation, the apparent species specificity, and the availability of studies at similar doses for other effects.

Liver Toxicity

Although liver toxicity is frequently reported in animal studies with TCE, there is more limited evidence for liver effects in humans (ATSDR 1997, Davidson and Beliles 1991). Liver toxicity has been reported in some cases of exposure to high concentrations resulting in death. Some studies report liver damage following lower exposures, while a controlled exposure (200 ppm) and several case studies of workers report no alterations of liver function.

Liver effects of exposure to inhaled trichloroethylene have been studied in several species (Kjellstrand *et al.* 1981, Prendergast *et al.* 1967, Nakajima *et al.* 1988, Kimmerle and Eben 1973). No gross pathological liver effects were observable in several species (rats, guinea pigs, rabbits, dogs) exposed to 730 ppm (8 h/d, 5 d/wk, 6 wk) and histochemical studies of enzyme activities in liver tissues of three rats showed no changes (Prendergast *et al.* 1967). As with oral studies, altered LW/BW ratio was frequently reported without determining other more direct measures of liver toxicity (Kjellstrand *et al.* 1981, 1983a,b). A dose-response for LW/BW increases is reported for exposures of 37 - 300 ppm continuously for 30 days. Exposure to 150 ppm for 30 days showed that mice were much more sensitive to increased LW/BW than were either rats or gerbils (Kjellstrand *et al.* 1981, 1983a). This is similar to findings with oral exposure demonstrating the mice are more sensitive than rats (Stott *et al.* 1982, Elcombe *et al.* 1985). In addition, this study demonstrates that the effect in mice is largely, though not completely, reversed in the 30 days following exposure. A 14-week study (55 ppm for 8 hr/day) reported increased LW/BW but no other pathological changes in liver (Kimmerle and Eben 1973). Two studies also demonstrated that ethanol exposure increased liver toxicity due to TCE exposures of 500 ppm or greater (Nakajima *et al.* 1988, Okino *et al.* 1991). This finding reflects, in whole or part, increased TCE metabolism due to induction of cytochrome P450 2E1 levels by ethanol. As for oral exposure, increased LW/BW ratio is not a direct measure of liver toxicity but may be used in this case as an early indicator. The dose-response data of Kjellstrand *et al.* (1983a) were evaluated quantitatively.

Alterations in activity of δ-aminolevulinic acid dehydratase (a biosynthetic enzyme in the heme pathway) in liver, erythrocytes, and bone marrow were determined in rats following inhalation of TCE (Koizumi *et al.* 1984, Fujita *et al.* 1984). Dose-dependent decreases were found following exposures to 50, 400, and 800 ppm. These alterations led to other changes in the relevant biochemical pathways such as increases in δ-aminolevulinic acid synthase in liver and increased excretion of in δ-aminolevulinic acid, but no liver injury or hematological changes were observed. The toxicological significance of these findings is unclear so they will not be evaluated further.

Methods for Selection of Critical Studies

This report attempts to present the full range of options, but ultimately also reflects the best professional judgments of its authors as to where to focus their efforts. Therefore, efforts have been made to document these judgements so that, while others may agree or disagree, the fact that those choices were made is explicit and the reasoning is presented.

Potential critical studies were identified in several ways. Existing literature reviews were used extensively (Gist and Burg 1995; Davidson and Beliles 1991), particularly the Toxicological Profile for Trichloroethylene (ATSDR 1997) which includes reports of the doses used and lists key studies for a wide range of effects. An analysis of oral toxicity studies had been prepared previously with a similar focus on risk assessment options (Barton and Das 1996). Computerized searching of Medline identified newer literature. Based upon these sources, original literature was obtained for review to determine its suitability to serve as the basis for developing noncancer dose-response values.

Selection of the critical study cannot simply be based upon the doses used in the study, because different choices in methods (e.g. NOAEL vs. BMD) and uncertainty factors can alter which study results in the lowest dose-response value. The choice was made, however, to evaluate the toxicological significance of studies prior to calculating the dose-response values rather than taking every study through the quantitative analysis. These judgments are documented below.

QUANTITATIVE DOSE-RESPONSE ANALYSIS

Each study previously identified as a potential critical study was evaluated using one or more methods for developing toxicity values (Barton *et al.* 1998, U.S. EPA 1994). These methods include identification of NOAELs or LOAELs, calculation of benchmark doses (BMDs), estimation of internal dose metrics using physiologically based pharmacokinetic (PBPK) modeling, and selection of uncertainty factors. Regardless of the methods used, however, a consistent process is required to allow comparisons across methods (Figure 1).

All the studies used are based upon results in laboratory animals, so the first step is to evaluate the animal data. The analysis is first carried out using exposure doses and NOAELs or BMDs. Next, mode of action information is incorporated describing both pharmacokinetics and pharmacodynamics, though data on the latter are generally very limited. The next step is extrapolation to humans, again either with exposure doses or incorporating internal dose metrics and available pharmacokinetic and mode of action information. When internal dose metrics are used, the corresponding human exposure dose is then estimated.

The proper point in the process for application of uncertainty factors (UF) is an issue when internal dose metrics are estimated. One perspective is that UFs have developed from rules of thumb based upon toxicological experience with exposure dose-response data and, therefore, they should be applied to exposure conditions in animals or humans regardless of whether internal dose metrics are calculated. Another perspective is that UFs should be applied to the animal dose metric prior to extrapolation to humans because the adjustments are meant to estimate the NOAEL that would have been obtained if all the needed data were available (i.e. from chronic exposures at low enough doses). If there were significant nonlinearities in metabolism, for instance, application of the UF to the exposure dose would not change the internal dose metric proportionately. The human model for TCE is essentially linear over a very wide dose range (see Table 4) so the issue can be sidestepped here. However, the underlying issues relating to extrapolation of pharmacokinetics s and pharmacodynamics across species are important ones that need further consideration.

Pharmacokinetic Model: Description and Use

The model of Clewell *et al.* (1998) briefly described in Clewell *et al.* (1995) was used to generate estimated internal or external doses. This model has been used to describe the pharmacokinetics in mice, rats, and humans of TCE and its major or toxicologically important metabolites, trichloroethanol (TCOH), trichloroacetate (TCA), dichloroacetate (DCA), and dichlorovinylcysteine (DCVC). It describes both oral and inhalation exposure regimens. A

range of possible dose metrics can be described with this model including: area under the curve (AUC) in blood for TCE (designated AUCTCE), AUC in blood for TCA (designated AUCTCA), TCOH (designated AUCTCH) and total metabolites normalized to body weight (designated AMET/BW). Other dose metrics can also be estimated such as peak concentrations for TCE in venous blood (designated CVTCE), TCE in arterial blood (designated CATCE), TCA (designated CTCA) and TCOH (designated CTCOH). The model was exercised using the Advanced Continuous Simulation Language (ACSL, Mitchell & Gauthier Associates, Concord, MA).

Several notable kinetic differences between the species have been observed in the experimental literature and are described in this model. Mice metabolize TCE much more effectively than do either rats or humans. The two major metabolites of TCE are TCA and TCOH. In humans, TCOH appears to undergo extensive enterohepatic recirculation of its glucuronide conjugate resulting in a much longer half-life for TCOH in humans compared to the rodents. Because TCOH can be metabolized back to TCA, this also results in a much longer half-life for TCA in humans compared to rodents.

As shown in Table 4, the human model was essentially linear for all dose metrics evaluated over a wide range of exposure doses. The human exposures were inhalation and drinking water, both of which were modeled as continuous exposures. Only above 100 ppm or 10 mg/kg/day in drinking water do the pharmacokinetics become nonlinear. The internal dose metric, therefore, can be converted to an exposure dose by multiplying by the appropriate conversion constant. For risk assessment purposes the meaning of the dose metric (assuming the appropriate dose metric was selected) and exposure dose in terms of cross-species response must also be considered. That is, the dose metric was obtained that resulted in no effect, the NOAEL exposure dose, or a specified effect level, the BMD, in rodents. The question then is how much effect would result from the same internal dose metric in humans? In a few cases there were too limited data to try to address this pharmacodynamic issue, e.g. Liver and neurotoxicity with TCE. In other cases there were no data so a default assumption must be used. The proposals for the default are that humans are equally sensitive to rodents for a given dose metric level (i.e. No

TABLE 4: HUMAN DOSE METRICS ESTIMATED FROM THE HUMAN PBPK MODEL

		Daily Average for:			Peak Values for:				
BW (kg)	DOSE	AUCTCE mg*hr/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	AMET mg/L	CVTCE mg/L	CTCA mg/L	CTCOH mg/L	CATCE mg/L
	Oral								
70	0.0001	1.9E-05	4.8E-02	6.5E-04	8.7E-05	9.2E-07	2.0E-03	2.7E-05	8.0E-07
70	0.001	1.9E-04	4.8E-01	6.5E-03	8.7E-04	9.2E-06	2.0E-02	2.7E-04	8.0E-06
70	0.01	1.9E-03	4.8E+00	6.5E-02	8.7E-03	9.1E-05	2.0E-01	2.7E-03	7.8E-05
70	0.1	1.9E-02	4.8E+01	6.5E-01	8.7E-02	9.0E-04	2.0E+00	2.7E-02	7.9E-04
70	1	1.9E-01	4.8E+02	6.6E+00	8.7E-01	9.1E-03	2.0E+01	2.8E-01	7.9E-03
70	10	2.0E+00	5.3E+03	7.5E+01	8.6E+00	9.8E-02	2.2E+02	3.1E+00	8.5E-02
45	0.0001	1.7E-05	4.3E-02	5.8E-04	8.7E-05	8.2E-07	1.8E-03	2.4E-05	7.2E-07
45	0.001	1.7E-04	4.3E-01	5.8E-03	8.7E-04	8.2E-06	1.8E-02	2.4E-04	7.2E-06
45	0.01	1.7E-03	4.3E+00	5.8E-02	8.7E-03	8.2E-05	1.8E-01	2.4E-03	7.2E-05
45	0.1	1.7E-02	4.3E+01	5.8E-01	8.7E-02	8.1E-04	1.8E+00	2.4E-02	7.0E-04
45	1	1.7E-01	4.3E+02	5.9E+00	8.7E-01	8.2E-03	1.8E+01	2.5E-01	7.1E-03
45	10	1.8E+00	4.7E+03	6.6E+01	8.6E+00	8.7E-02	2.0E+02	2.7E+00	7.5E-02
	Inhalation								
70	0.0001	5.6E-05	2.3E-02	3.1E-04	4.2E-05	2.0E-06	9.6E-04	1.3E-05	2.4E-06
70	0.001	5.6E-04	2.3E-01	3.1E-03	4.2E-04	2.0E-05	9.6E-03	1.3E-04	2.4E-05
70	0.01	5.6E-03	2.3E+00	3.1E-02	4.2E-03	2.0E-04	9.6E-02	1.3E-03	2.4E-04
70	0.1	5.6E-02	2.3E+01	3.1E-01	4.2E-02	2.0E-03	9.6E-01	1.3E-02	2.4E-03
70	1	5.6E-01	2.3E+02	3.2E+00	4.2E-01	2.0E-02	9.7E+00	1.3E-01	2.4E-02
70	10	5.7E+00	2.4E+03	3.4E+01	4.2E+00	2.0E-01	1.0E+02	1.4E+00	2.4E-01
70	100	6.1E+01	3.4E+04	5.4E+02	3.9E+01	2.2E+00	1.4E+03	2.3E+01	2.5E+00
45	0.0001	5.6E-05	2.3E-02	3.1E-04	4.7E-05	2.0E-06	9.6E-04	1.3E-05	2.4E-06
45	0.001	5.6E-04	2.3E-01	3.1E-03	4.7E-04	2.0E-05	9.6E-03	1.3E-04	2.4E-05
45	0.01	5.6E-03	2.3E+00	3.1E-02	4.7E-03	2.0E-04	9.6E-02	1.3E-03	2.4E-04
45	0.1	5.6E-02	2.3E+01	3.1E-01	4.7E-02	2.0E-03	9.6E-01	1.3E-02	2.4E-03
45	1	5.6E-01	2.3E+02	3.2E+00	4.7E-01	2.0E-02	9.7E+00	1.3E-01	2.4E-02
45	10	5.7E+00	2.4E+03	3.4E+01	4.6E+00	2.0E-01	1.0E+02	1.4E+00	2.4E-01
45	100	6.1E+01	3.4E+04	5.4E+02	4.3E+01	2.2E+00	1.4E+03	2.3E+01	2.5E+00

further interspecies adjustments are required) or that humans are more sensitive (i.e. additional interspecies adjustments are required). This issue will be addressed further in the discussion of the selection of uncertainty factors for the various effects.

Benchmark Dose Methods

Three different kinds of data were used in BMD analyses: quantal, continuous, and nested quantal (i.e. litter) data. For each type of data, different methods were used. The quantal data were evaluated using two programs, THRESH and THRESHW (ICF Kaiser, KS Crump Group, Ruston, LA). THRESH fits a polynomial model and THRESHW fits a Weibull model to the data (Crump 1984). The continuous data were analyzed with BENCH_C (ICF Kaiser, KS Crump Group, Ruston, LA) which fits the Power and Weibull models (Crump 1995). In addition, a quadratic model for analyzing continuous data was used for comparative purposes with the liver data of Buben and O'Flaherty (1985) (Kodell and West, 1993). This program was generously provided by Dr. R. L. Kodell (National Center for Toxicology Research, Jefferson, AK). Finally, the litter incidence data were analyzed using TERAMOD and TERALOG (ICF Kaiser, KS Crump Group, Ruston, LA) (Allen *et al.* 1994); these models account for possible extra-binomial variation associated with nested responses. No matter what the type of data, the models considered express probability of response as a function of dose.

The BMD analysis for each data set included using two models to estimate the maximum likelihood estimate (MLE) or "best fit" and its statistical lower bound (BMDL) for either a 10% or 5% bench mark risk (BMR). Lower values of BMR result in lower estimates of the MLE and BMDL. For continuous data, the region of the distribution of control responses that was considered abnormal is defined by the parameter P_0. Three values of P_0 were used, 0.05, 0.01, and 0.001. As the value of P_0 decreases, a smaller portion of the control distribution is considered abnormal and the acceptable variation for the continuous endpoint (e.g. LW/BW) is larger. Thus, lower values for P_0 result in higher estimates for the MLE and BMDL.

There currently are limited scientific or policy justifications for choosing between alternative values of P_0 so additional risk assessment guidance is needed in order to implement this

approach. One argument for the use of $P_0 = 0.05$ is that this is the value traditionally used in human clinical chemistry studies for separating abnormal from normal results. It might be anticipated that the variability for humans would be greater than for inbred rodents, so it would be appropriate to use a lower value for P_0. In this report, we have chosen to use $P_0 = 0.01$ when reporting results in the text. Results for other values of P_0 are presented in Tables A2, A3, A5, and A6.

Concentration-Time Adjustments and Uncertainty Factors

The standard methods for developing RfDs and RfCs from exposure conditions are similar when the chemical is considered a nonreactive gas in the RfC methodology (U.S. EPA, 1994). First, adjustments are made from intermittent to continuous exposure. Then, the Human Equivalent Concentration (HEC) for a NOAEL is calculated using the approach for a Category 3 gas causing extrarespiratory effects. In this approach, the adjusted NOAEL is multiplied by the ratio of blood air partition values in animals to humans or by 1.0 if the value for animals is greater than that for humans, unless a PBPK model is used; for TCE a value of 1.0 is used. UFs are applied.

Generally, the exposure dose or concentration is adjusted assuming that the concentration-time product ($C \times T$) is constant if dosing was not daily (oral) or continuous (inhalation). When dose metrics were calculated using a PBPK model, the average daily AUC was estimated (i.e. averaged over periods lacking exposure). If peak blood or tissue concentrations were used, they were not averaged.

The UFs used were those described for RfD and RfC derivation to account for uncertainty - for use of LOAEL rather than a NOAEL (referred to as L), for use of a study of less than chronic duration (referred to as S), for animal to human extrapolation (referred to as A), for human variability (referred to as H) and for database limitations (referred to as D). Each UF usually has a value up to 10 though this appears to be overly conservative when multiple factors are used (Dourson and Stara 1983). Therefore, policy choices have been made to limit the total UFs of 10 when four or five UFs are used; four UFs would result in a total UF of 3000, rather than 10,000 (U.S. EPA 1994).

Uncertainty factors and C x T adjustments are semiquantitative tool for extrapolating information. As such, they are additional methods used within the exposoure:dosimetry:mode-of-action:response framework. This is particularly apparent for those extrapolating to chronic exposure from shorter duration studies (S) and interspecies extrapolation (A), which are critically dependent upon the mode of action and underlying pharmacokinetics. The appropriate selections for the interspecies UF when pharmacokinetics has been used is not entirely clear. Early suggestions were that the UF was based upon pharmacokinetic differences between animals and humans. More recently it has been suggested that the default 10-fold UF can be apportioned into pharmacokinetic (delivered dose) and pharmacodynamic (related to target tissue sensitivity) factors (Renwick 1993). The RfC methodology specifies that following the adjustments for dosimetry, the UF for animal to human extrapolation will be 3, rather than 10 (U.S. EPA 1994). In this analysis, when pharmacokinetics is incorporated in RfD development the default in the absence of pharmacodynamic information will be 3 for A, rather than 10 (US EPA 1994). Other UFs fall partially within the organizational framework, such as estimating human variability (Barton *et al.* 1996), though the general lack of information in this area makes this practically a policy choice. The database uncertainty factor is primarily a policy response to data limitations and essentially falls outside the framework.

ORAL STUDIES – DOSE-RESPONSE BASED UPON EXPOSURE DOSE METRIC

Six studies reporting four different toxic effects were evaluated quantitatively as shown in Table 1. The NOAELs, LOAELs, and BMDs for each study are reported in Table 5.

Exposure Dose-Based NOAELs and RfDs

Eye Malformation:

Comparison of means found the number of affected rats in only the highest dose group, 1125 mg/kg/day, to be statistically different from the controls (Narotsky *et al.* 1995). Trend testing to find the no-statistical-significance-of-trend (NOSTASOT) dose, found a LOAEL of 101 mg/kg/day and a NOAEL of 32 mg/kg/day (Barton and Das, 1996). These developmental exposures were performed daily so no dose averaging adjustments were made. The NOAEL was

divided by a total UF of 100 (10 for H and 10 for A) to give an RfD of 0.3 mg/kg/day (see Table 11).

TABLE 5: NOAELS, LOAELS AND BMDS FOR ORAL STUDIES BASED UPON EXPOSURE DOSES

Effect	Study citation	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	MLE[a] (mg/kg/day)	BMDL[b] (mg/kg/day)
Eye malformations	Narotsky *et al.* 1995	32	101	777, 751[d]	505, 501[d]
LW/BW	Tucker *et al.* 1982	18	217	N.D.	N.D.
LW/BW	Buben and O'Flaherty 1985	N.D.	100	20, 489[c]	14, 341[c]
LW/BW	Berman *et al.* 1995	N.D.	50	3309, 1403[c]	742, 650[c]
Immune functions	Sanders *et al.* 1982	200	400	N.D.	N.D.
Kidney toxicity	Maltoni *et al.* 1986	50	250	105[e]	70[e]

N.D. Not determined in study.
[a]Maximum likelihood estimate for 10% likelihood of response.
[b]Lower bound estimate for 10% likelihood of response.
[c]BMD estimates for $P_0 = 0.05$, and BMR = 0.1; Results for Weibull and Power models. Unadjusted to continuous exposure.
[d]BMD estimates for BMR = 0.1; Results for TERALOG and TERAMOD models.
[e]BMD estimates for BMR = 0.1; Results for Polynomial Quantal model.

Liver to Body Weight Ratio:

The six-month drinking water study reported a LOAEL of 217 mg/kg/day in male mice and a NOAEL of 18 mg/kg/day (Tucker *et al.* 1982). The LOAEL and NOAEL in female mice were 793 and 437 mg/kg/day. The drinking water study used continuous exposure so no dose averaging adjustment was performed (Tucker *et al.* 1982). Changes in LW/BW are being interpreted as an early event in the toxicity process and a sensitive indicator of potential liver

effects. Therefore, no adjustments for the duration of exposure (S) should be made regardless of the study duration. The NOAEL of 18 mg/kg/day was divided by 100 for H (10) and A (10) to give an RfD of 0.2 mg/kg/day.

Corn oil gavage exposure to TCE for six weeks (5 days/week) resulted in statistically significant increases in LW/BW in mice at all doses, so the LOAEL was 100 mg/kg/day (Buben and O'Flaherty 1985). The mouse corn oil gavage study used 5 day/week dosing so the LOAEL of 100 mg/kg/day was adjusted by U.S. EPA to 71 mg/kg/day. This adjusted LOAEL was divided by 300 for L (3), H (10) and A (10) to give an RfD of 0.2 mg/kg/day. The value of 3 for L is based upon the LOAEL being minimal, the effect was minor and the size of the change was small, just 12%.

A 14-day corn oil gavage exposure to TCE resulted in increases in LW/BW in rats at all doses (Berman *et al.* 1995). The increase in average LW/BW at 50 mg/kg/day was only 7%, so it would be considered a minimal LOAEL (U.S. EPA, 1994). The rat corn oil gavage study used 14 daily exposures so no dose averaging adjustment was made (Berman *et al.* 1995). The LOAEL of 50 mg/kg/day was divided by a total UF of 300 (3 for L, 10 for H and 10 for A) to give an RfD of 0.2 mg/kg/day (see Table 11).

Immunotoxicity:

Impaired immune functions were observed in rats, particularly in females, exposed to TCE in drinking water for four months (Sanders *et al.* 1982). The variability of responses makes the interpretation of the various assays important. The antibody-forming (plaque-forming) cell assay has been shown to be highly correlated with immunotoxicity (Luster *et al.* 1992a, b). It had a LOAEL of 400 mg/kg/day in female mice at four months and male mice at six months; the NOAELs were 200 mg/kg/day. The delayed hypersensitivity (DHS) assay has been shown to be less correlated individually, but highly correlated with immunotoxicity when paired with the plaque-forming assay (Luster *et al.* 1992a, b). The DHS assay had a LOAEL of 20 mg/kg/day in female mice at four months compared to the vehicle treated control, but there was a large difference between the vehicle treated control and naive control. At six months, the LOAEL was 800 mg/kg/day and the NOAEL was 400 mg/kg/day in females. No effects were seen in males.

Taken together, these assays are supportive of a LOAEL of 400 mg/kg/day and a NOAEL of 200 mg/kg/day.

The drinking water study used continuous exposure so no dose averaging adjustment was made (Sanders *et al.* 1982). The NOAEL of 200 mg/kg/day was divided by a total UF of 100 (10 for H and 10 for A) to give an RfD of 2 mg/kg/day (see Table 11). No adjustments for the duration of exposure (S) were made because there appears to be a greater effect at 4 months than at 6 months.

Kidney Toxicity:

Kidney toxicity was reported in the Maltoni *et al.* (1986) study in male Sprague-Dawley rats dosed 250 mg/kg, but not 50 mg/kg for 5 days/week for 52 weeks. The animals were observed until natural death. The NOAEL of 50 mg/kg/day was multiplied by 5/7 to adjust to daily dosing giving a NOAEL of 36 mg/kg/day. The study dosed for a significant portion of lifetime so no adjustment was made for duration. It is unclear, however, if repair of kidney toxicity might have occurred and reduced the incidence during the year following dosing which was intended to allow expression of tumors. The NOAEL was divided by 100 for H (10) and A (10) to give an RfD of 0.4 mg/kg/day (see Table 11).

Exposure Dose-Based BMDs and RfDs

The BMDs presented in the text are for a 10% risk of response (BMR = 0.1). Data at a 5% risk level are presented in the tables in Appendix A. For continuous variables (e.g. LW/BW) the portion of the control distribution considered abnormal was selected as 1% for values in the text ($P_0 = 0.01$). Alternative calculations using 0.05 and 0.001 are reported in the tables in Appendix A. As described in the methods, the maximum likelihood estimate is noted as the MLE, while the lower bound on this estimate is designated the BMDL.

Eye Malformation:

The BMDs obtained with the two models were very similar using exposure doses (Figure 2). The MLE was 777 mg/kg/day with TERALOG and 751 with TERAMOD. The BMDLs were 505 and 501 mg/kg/day with TERALOG and TERAMOD, respectively. The BMDLs are much higher than the NOAEL because the observed response was so small. Dividing the BMDL by 100, 10 for H and 10 for A, gives an RfD of 5 mg/kg/day.

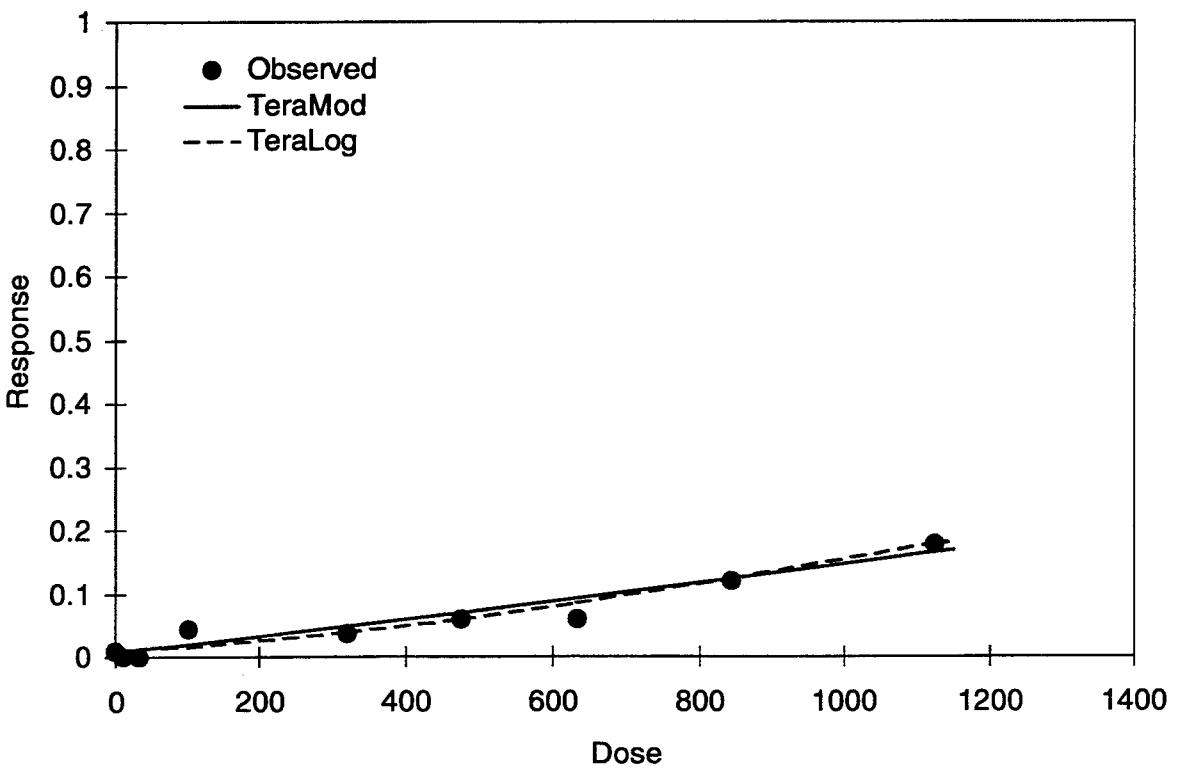

Figure 2: Eye Malformations - Maximum Likelihood Fits to Exposure Doses (BMR = 0.1) (Narotsky *et al.* 1995).

Liver to Body Weight Ratio:

No BMD was estimated for the drinking water data because the values of LW/BW were not reported (Tucker *et al.* 1982). The MLEs obtained with the exposure concentrations from both the mouse (Buben and O'Flaherty 1985) and the rat (Berman *et al.* 1995) studies were very dependent upon the choice of model (see Figure 3a). For the mouse, the Weibull model predicts a MLE of 20 mg/kg/day and the Power model predicts 489 mg/kg/day. Similarly the BMDLs for

the mouse are also very different depending upon the model used (14 and 341 mg/kg/day for the Weibull and Power models, respectively). For the rat, the Weibull model predicts a MLE of 3309 mg/kg/day and the Power model predicts 1403 mg/kg/day. By contrast, the BMDLs are more similar, 742 and 650 mg/kg/day for the Weibull and Power models, respectively. These differences between models reflect the difficulties the models have fitting these data.
A quadratic model was also used to fit the mouse data of Buben and O'Flaherty (1985) as illustrated in Figure 3a (Kodell and West 1993). This model appears to better fit the low dose data resulting in BMDs that fall in between those from the Weibull and Power models. For $P_0 = 0.01$ and BMR = 0.1, the MLE was 201 and the BMDL was 173 mg/kg/day. This fit arises, in part, because the program allows the parameters to take negative values. At high doses, the negative value of the parameter in the dose squared term results in a plateau and even slight decline in the predicted values. This does not appear particularly important for estimating the MLE and BMDL.

The BMDLs calculated from the mouse study using the Weibull, Power and quadratic models were multiplied by 5/7 giving 10, 244, and 124 mg/kg/day. Dividing these values by 100, 10 for H and 10 for A, gives RfDs of 0.1, 2, or 1 mg/kg/day. The wide variation in BMDL values makes it difficult to utilize this approach unless qualitative judgements are applied about the selection of the most appropriate model. The BMDLs for the rat study were divided by 100 to give RfDs of 7 mg/kg/day for both models.

Immunotoxicity:

The BMD method was not used for this study (Sanders *et al.* 1982), which included three assays with different dose-responses, because it was felt that no single assay should be used alone to determine the dose-response relationship.

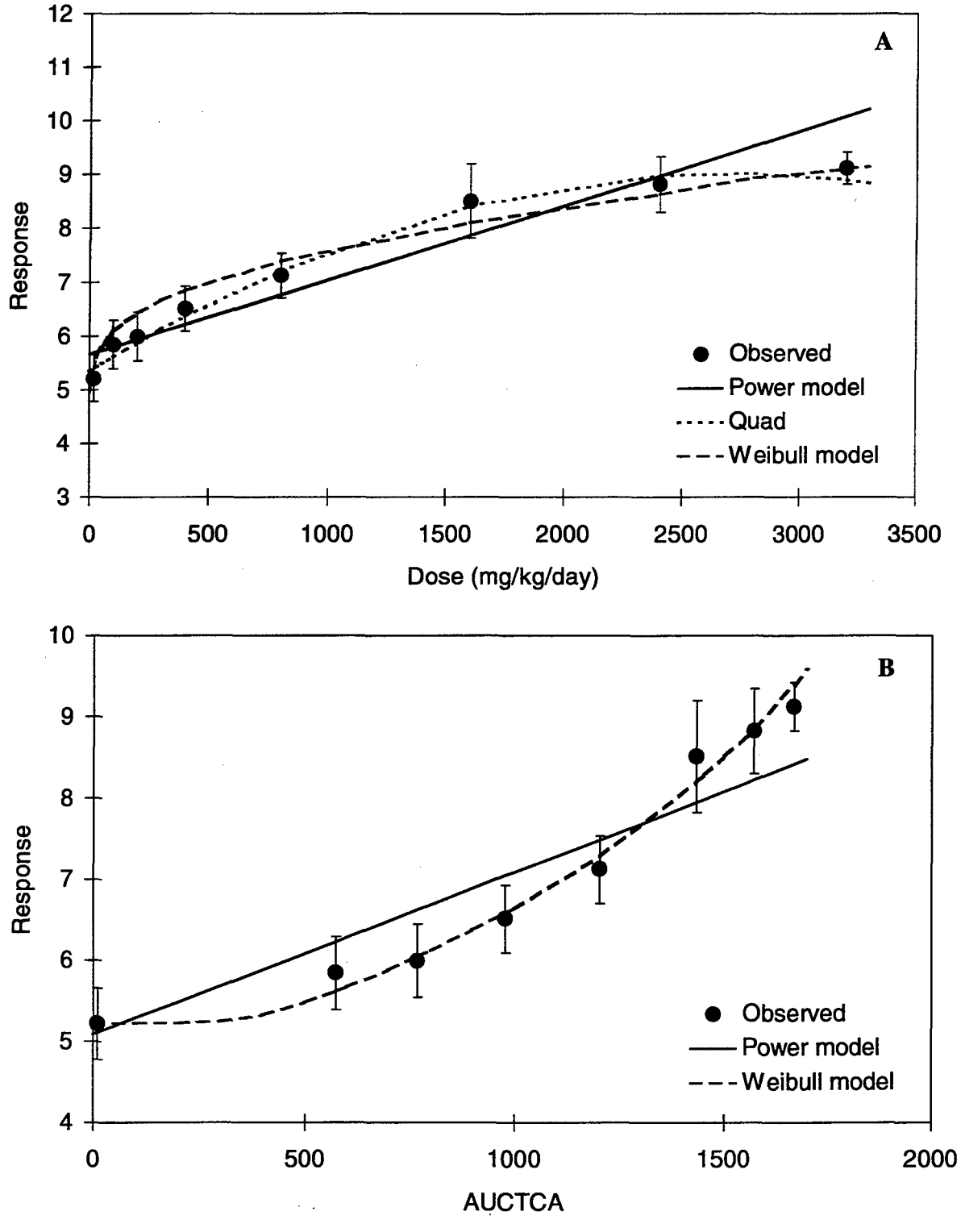

Figure 3: Liver effects. Model fits to LW/BW using alternate dose metrics: (A) Exposure doses P_0=0.01), (B) AUCTCA (P_0=0.01, BMR=0.1) (Buben and O'Flaherty 1985).

Kidney Toxicity:

This data set is only marginally acceptable for the BMD method because there is only a single positive dose (Figure 4). However, it was evaluated because there is reason to believe that a response of nearly 100% is potentially obtainable based upon the inhalation data and oral studies in five other rat strains. The 46.7% response at 250 mg/kg is a valid estimate of the percent of total response. The polynomial model appears to provide a more reasonable fit, estimating the MLE as 105 mg/kg/day and the BMDL as 70 mg/kg/day. The BMDL was adjusted by 5/7 to 50 mg/kg/day. Dividing by 100, 10 for H and 10 for A, gives an RfD of 0.5 mg/kg/day (see Table 11).

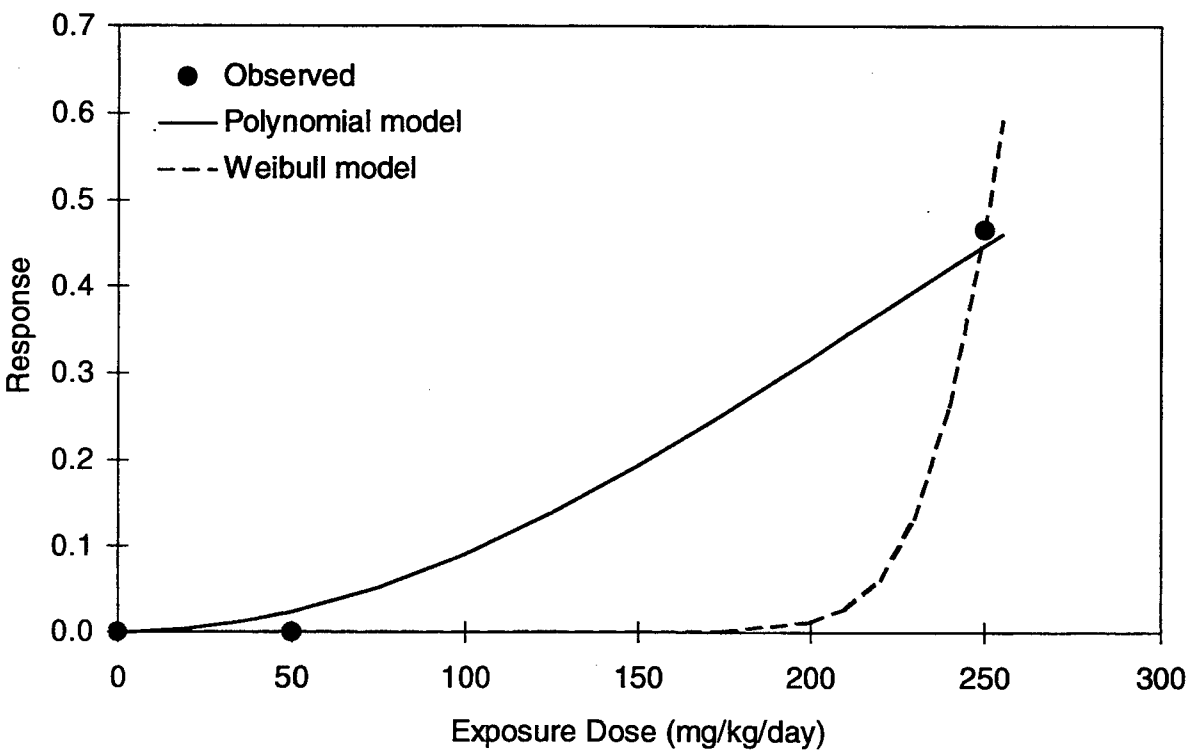

Figure 4: Kidney effects. Model fits using oral exposure (BMR=0.1) (Maltoni *et al.* 1986).

ORAL STUDIES - INCORPORATING PHARMACOKINETICS AND PHARMACODYNAMICS

Studies to be evaluated as potential critical studies were simulated with the PBPK model to obtain estimated internal dose metrics. The values for the dose metrics at the NOAELs or LOAELs were determined. In addition, the internal dose metrics were used to calculate BMDs (expressed in units of the internal dose metrics). To obtain an RfD for human, the internal animal doses are divided by the appropriate uncertainty factors and the human PBPK model is used to equate the internal human dose metrics to a human exposure dose. Because human pharmacokinetics for TCE and its metabolites are linear over a wide range of doses, the ordering of these two steps (i.e., application of uncertainty factors and conversion of internal to exposure dose) does not matter.

Selection of Pharmacokinetic Dose Metrics and Pharmcodynamic Adjustments

Eye Malformations:

No mode of action hypotheses have been proposed for this endpoint that would assist in selecting the appropriate internal dose metric. The teratogenicity studies with TCA and DCA both report increases in microphthalmia and anophthalmia (Smith *et al.* 1989, 1992), while no data were available for CH or TCOH. This suggests that one or both of the acids may contribute to the effects seen with TCE. Comparison of the AUCTCA at doses of TCE and TCA that produced about 10% incidence of eye malformations in pups found drastically different results. The AUCTCA estimated for the TCA dose of 1200 mg/kg/day was about 20,000 mg*h/L, while TCE at 1125 mg/kg/day produces an AUCTCA of 405 mg*h/L. This discrepancy raises doubt that the levels of TCA produced from TCE would be adequate to cause the effect. Two limitations of this analysis do not permit exclusion of TCA as a causative agent. First, the strains of rats were different. Second, this analysis assumes circulating maternal levels of TCA are predictive of fetal tissue exposure. TCE may be metabolized in the fetus, although cytochrome P450 2E1 levels are low prior to birth and increase greatly shortly after (Cresteil 1998).

Although most toxicities associated with TCE are thought to result from metabolites, it is possible that changes in membrane characteristics due to TCE, itself, are responsible for the developmental effects because development is highly dependent upon cell-cell communications. It is also undetermined if the effects result from the concentration of chemical present or a time integrated measure of dose, such as area under the concentration curve. Finally, in the absence of models for both the developing rat and human fetus, only dose metrics in the maternal blood perfusing the placenta were estimated. In the absence of strong mode of action hypotheses, AUCs in blood in the maternal rats were estimated for TCE (AUCTCE), and the two major metabolites, TCA (AUCTCA) and TCOH (AUCTCH). In additions, dose metrics for peak concentrations are reported for TCE (CVTCE, CATCE), TCA (CTCA), and TCOH (CTCOH). These dose metrics were obtained by running the PBPK model for 240 hours and dividing the total dose metrics for that time by 10 days to obtain the daily average AUC.

Only AUCTCA was used as a dose metric for BMD analysis. This dose metric was selected because it is generally the most conservative for interspecies extrapolation with TCE.

In the absence of any mode of action information or models for the eye malformations, the default assumption was made that humans are 3-fold more sensitive than animals for the pharmacodynamic processes. In addition, a 10-fold UF for H was used. Given the lack of good mode of action information, this analysis was undertaken to provide a sense of what results might be obtained if one of the potential dose metrics were appropriate and particularly to guide planning of additional mode of action studies.

Liver to Body Weight Ratio:

Alterations in the liver are believed to be due to metabolites. It was shown by Buben and O'Flaherty (1985) that use of total urinary metabolites linearized their LW/BW data, so total metabolites normalized to (divided by) body weight (referred to as AMET) was one potential dose metric. This dose metric is consistent with effects resulting from metabolism of TCE with no adjustments for the pharmacokinetics of the individual metabolites (i.e. short-lived versus long-lived etc.). However, analysis of the data on LW/BW and enzyme changes following

dosing with TCE and perchloroethylene suggest that AMET is not a satisfactory dose metric because it would predict that TCA from perchloroethylene (60 - 90% of urinary metabolites) was more potent than TCA from TCE (about 10% of urinary metabolites) (Buben and O'Flaherty 1985).

Oral exposure to TCA produces increased LW/BW, peroxisome proliferation, and other effects associated with TCE, so it is an active metabolite (DeAngelo *et al.* 1989, Sanchez and Bull 1990, Larson and Bull 1992b, Styles *et al.* 1991). One potential dose metric for TCA is area under the blood concentration curve (AUCTCA). This might be an appropriate dose metric for liver changes involving receptor-mediated processes such the pleiotropic effects occurring with peroxisomal proliferators. Although peroxisomal proliferation is the most obvious change observed in rodents, other changes in cellular regulation associated with peroxisome proliferator-activated receptor (PPAR) activity may be more relevant to humans. The dose metric (AUCTCA) was obtained by running the PBPK model for 336 or 1008 hours and dividing the total dose metrics for that time by 14 or 42 days, for the rat (Berman *et al.* 1995) and mouse (Buben and O'Flaherty 1985) studies, respectively, to obtain the daily average AUC.

Finally, several other dose metrics might be relevant for the effects in mice or rats including those for DCA. There was a lack of data supporting human DCA production from TCE, so it was not possible to use PBPK modeling to obtain an equivalent human dose even if a dose metric was obtained for mice. In addition, estimates of DCA production in mice have been excessively high due to analytical chemistry artifacts (Ketch *et al.* 1996). This raises a difficulty because it would be desirable to know the extent to which DCA is involved in the response in mice in order to determine if extrapolation from other dose metrics is appropriate. There were also no data for TCOH and liver effects, though some effects would be expected because it can be metabolized to TCA.

For liver effects there is a database from which to develop information about the mode of action in animals and humans. Only information for TCE or its metabolites was considered here, but

there were data on pharmaceutical agents that cause peroxisome proliferation that could be considered to improve the extrapolation to humans.

Trichloroethylene has been tested in mice, rats, gerbils, guinea pigs, rabbits, and dogs by oral or inhalation exposures. It produces limited noncancer liver toxicity in any of these species until doses approaching the LD_{50} are reached. Similarly, the limited available data indicate that TCE is not a potent liver toxicant in humans as would appear to be predicted by AUCTCA if equal or greater pharmacodynamic sensitivities were assumed.

Because LW/BW changes are believed to involve the PPAR, extrapolation of the response to humans is not quantitatively straightforward. One approach to estimating the interspecies pharmacodynamic extrapolation was to compare rats and mice. Data for increases in LW/BW and palmitoyl-CoA oxidase activity (a marker for peroxisomal proliferation) following drinking water exposure to TCA for 14 days showed rats to be much less responsive than mice based upon estimated AUCTCAs (DeAngelo *et al.* 1989). Rats were found to be more sensitive than mice for palmitoyl-CoA oxidation following 10 days dosing with TCA in corn oil. When AUCTCA is estimated for this study, the rats are about 1.5 times more responsive. DeAngelo *et al.* (1989) found that corn oil increased response in rats compared to an aqueous vehicle. Therefore, there are two issues for extrapolating to humans, their sensitivity relative to rodents and the effects of corn oil in the rodents versus drinking water in humans. It is likely that humans are more like rats than mice, so these data do not support the standard assumption that humans are more sensitive than the most sensitive rodents. Analysis of the interspecies extrapolation for other peroxisomal proliferators, particularly the hyperlipidemic drugs would assist in further determining the appropriate interspecies extrapolation. These data indicate that the value of the uncertainty factor for interspecies extrapolation should be no greater than 1 and potentially less than that. Finally, the value for H of 10 was used but this again may be higher than appropriate. The available molecular and pharmacological data have not identified humans with a fully active PPAR as found in the mouse. Therefore, the mouse appears to be a sensitive species.

Immunotoxicity:

Pharmacokinetic modeling was not undertaken for this endpoint. There was an absence of hypotheses for the mode of action of TCE for these effects, so all-possible dose metrics could be considered possible candidates. The lack of strong dose-response data made it impossible to potentially exclude dose metrics based upon the correlation with response data limiting the utility of pharmacokinetic modeling. Finally, the LOAELs for other endpoints were lower so this study was unlikely to serve as the basis for the RfD.

Kidney Toxicity:

This toxicity is believed to develop from metabolites formed through the glutathione conjugate pathway (Goeptar *et al.* 1995). Therefore, the PBPK model was used to estimate a dose metric for DCVC in the kidney (referred to as KTOX). KTOX represents the total production of the thioacetylating intermediate from DCVC divided by the volume of the kidney. The daily value for KTOX was obtained averaged over a one-week (7-day) period. Limited information is available about the pharmacodynamic processes involved in the kidney toxicity. Both mice and rats developed kidney toxicity in corn oil gavage assays and the mice appeared somewhat more sensitive (NCI 1976, NTP 1983, NTP 1988). In the absence of other information, the default assumption was made that humans are 3-fold more sensitive than animals for the pharmacodynamic processes leading to kidney toxicity.

Dose Metric-Based NOAELs and RfDs

Eye Malformations:

The dose metrics at the NOAEL exposure dose of 32 mg/kg/day were as follows: AUCTCE 0.62 mg*h/L, AMET 31 mg/L, AUCTCA 71 mg*h/L, AUCTCH 5.7 mg*h/L, CVTCE 0.46 mg/L, CTCA 4.6 mg/L, and CTCOH 2.7 mg/L (Table B-1). The UFs applied were 3 for A and 10 for H for a total of 30. The RfDs obtained in mg/kg/day were: AUCTCE 0.1, AMET 1, AUCTCA 0.005, AUCTCH 0.03, CVTCE 2, CTCA 0.008, CTCOH 0.3. The RfD based upon the exposure NOAEL was 0.3 mg/kg/day, so these dose metric-based values (0.005 – 1 mg/kg/day) span a range from 3-fold above to 60-fold below the exposure-based NOAEL. Clearly, assumptions about the appropriate dose metrics and associated pharmacodynamic

response make large differences because of the slow clearance of TCA and TCOH in humans compared to rats.

Liver to Body Weight Ratio:

The AUCTCA was 570 mg*h/L at the NOAEL of 18 mg/kg/day in the drinking water study with mice (Tucker *et al.* 1982). The exposure based LOAEL in the mouse corn oil gavage study was 100 mg/kg/day which corresponds to AUCTCA of 573 mg*h/L (Buben and O'Flaherty 1985). The exposure based NOAEL in the rat study was 50 mg/kg/day which corresponds to AUCTCA of 118 mg*h/L (see Tables B2 and B3) (Berman *et al.* 1995).

The dose metrics reported for these studies are average daily values so no further adjustments for intermittent exposure are required. The two mouse studies coincidentally had very similar values for AUCTCA despite their different exposure doses. This is due to the metabolic saturation and consequent exhalation of TCE following a single gavage dose while the continuous drinking water exposure is more completely metabolized. The mouse AUCTCA of 570 mg*h/L was divided by a total UF of 30 (3 for L, 10 for H and 1 for A) to estimate a human AUCTCA which was divided by 479 to convert to the exposure dose, giving an RfD of 0.04 (see Table 11). The rat AUCTCA of 118 mg*h/L was divided by the UF of 30 to estimate a human AUCTCA which was divided by 479 to convert to the exposure dose, giving an RfD of 0.008 (see Table 11).

Immunotoxicity:

Dose metric NOAELs were not developed for this endpoint (see Oral Studies - Incorporating Pharmacokinetics and Pharmacodynmics: Immunotoxicity).

Kidney Toxicity:

The Maltoni *et al.* (1986) study reported a NOAEL at the lowest dose. At this dose the value of KTOX was 85 mg/L. In the absence of data about pharmacodynamic sensitivity, the default value of 3 was used for A. Therefore, the total UF was 30 (10 for H and 3 for A) giving an RfD of 2 mg/kg/day (see Table 11).

Dose Metric-Based BMDs and RfDs

Eye Malformations:

There is very little information upon which to base the choice of a dose metric for the eye malformations. Because TCA also induced eye malformations and because it provides the most conservative estimate of acceptable exposure, AUCTCA was the only dose metric used for the BMD analysis. Values for the MLE and BMDL using AUCTCA were very similar using the two models, 3633 and 3023 using TERALOG and 3591 and 2924 using TERAMOD. The BMDL of 2924 was divided by a total UF of 30 (10 for H and 3 for A, see 3.5.2) and by 479 for conversion of human AUCTCA to an exposure dose to obtain the RfD of 0.2 (see Table 11). As shown previously, using AUCTCA results in among the lowest estimates of acceptable human doses because of the slow clearance of TCA in humans.

Liver to Body Weight Ratio:

The values of MLE and BMDL obtained with the Weibull and Power models for the dose metric, AUCTCA, are presented in Table 6 for the rat and mouse data (Figure 3b). By comparison with the BMDs for the exposure dose, the choice of model has much less effect when the internal dose metric is used. The mouse BMDL values of 551 and 196 mg*h/L were divided by a total UF of 10 (10 for H and 1 for A, see 3.5.2) and by 479 for conversion of human AUCTCA to an exposure dose to obtain the RfDs of 0.1 and 0.04. The rat BMDL values of 308 and 239 mg*h/L would also be divided by a total UF of 10 (10 for H and 1 for A) and by 479 to obtain the RfDs of 0.06 and 0.05 (see Table 11).

Immunotoxicity:

Dose metric BMDs were not developed for this endpoint.

Kidney Toxicity:

The MLE based upon KTOX is 673 mg/L and the BMDL is 230 mg/L. The BMDL was divided by a total UF of 30 (10 for H and 3 for A) and by 1.54 for conversion to the external human dose to estimate the RfD of 5 mg/kg/day (see Table 11).

TABLE 6: BMDS AND RFDS FOR LIVER EFFECTS USING INTERNAL DOSE METRICS

Dose metrics	AUCTCA - MLE (mg*h/l)	AUCTCA - BMDL (mg*h/l)	RFD-MLE[a] (mg/kg/d)	RFD-BMDL[a] (mg/kg/d)
Mouse				
Weibull	640	551	0.1	0.1
Power	250	196	0.05	0.04
Rat				
Weibull	613	308	0.1	0.06
Power	447	239	0.09	0.05

[a]RfD based upon UF = 10 and dividing by 479 to convert AUCTCA to human equivalent exposure dose

INHALATION STUDIES – DOSE RESPONSE BASED UPON EXPOSURE DOSE METRIC

Three studies reporting different toxic effects were evaluated quantitatively as shown in Table 3. The NOAELs, LOAELs, and BMDs for each study are reported in Table 7.

Exposure Dose-Based NOAELs and RfCs

Neurological Effects:

Measurements of electroencephalographic activity and heart rate were made during a 32-hr. period during the 2nd, 4th, and 6th weeks of exposures in freely moving male rats implanted with electrodes (Arito *et al.* 1994). The measurements were made during the 8-h exposure period and during a 22-h post-exposure. Three measures were derived from electroencephalographic measurements – wakefulness, slow-wave sleep, and paradoxical sleep; heart rate was measured independently. All four measures were statistically different from control levels at the lowest dose used, so the LOAEL was 50 ppm.

The LOAEL value was lowered to estimate continuous exposure from the 8 h/day, 5 d/week exposures resulting in an adjusted LOAEL of 12 ppm. Although this is a LOAEL it is for a

minimal effect so the UF was 3 instead of 10. The total UF was 100 (10 for H, 3 for A, and 3 for L) giving the RfC of 0.1 ppm (see Table 12).

Liver to Body Weight Ratio:

Increases in LW/BW were statistically significant in both male and female mice at all dose levels tested (Kjellstrand *et al.* 1983a). Therefore, the LOAEL was the lowest dose used, 37 ppm continuously for 30 days. This is a LOAEL for a minimal effect so the UF was 3 instead of 10. This value was divided by a total UF of 100 (10 for H, 3 for A, and 3 for L) giving the RfC of 0.4 ppm (see Table 12).

TABLE 7: NOAELS, LOAELS AND BMDS FOR INHALATION STUDIES BASED UPON EXPOSURE DOSES

Effect	Study citation	NOAEL (ppm)	LOAEL (ppm)	MLE[a] (ppm)	BMDL[b] (ppm)
Heart rate changes	Arito *et al.* 1994	N.D.	50	124, 279[c]	32, 122[c]
Wakefulness (Electroencephalographic)	Arito *et al.* 1994	N.D.	50	23, 253[c]	8,130[c]
Slow-Wave Sleep (Electroencephalographic)	Arito *et al.* 1994	N.D.	50	32, 161[c]	8, 78[c]
Paradoxical Sleep (Electroencephalographic)	Arito *et al.* 1994	N.D.	50	6478, 373[c]	223, 115[c]
LW/BW, female mice	Kjellstrand *et al.* 1983a	N.D.	37	44, 27[d]	32, 21[d]
LW/BW, male mice	Kjellstrand *et al.* 1983a	N.D.	37	11, 30[d]	5, 24[d]
Kidney toxicity	Maltoni *et al.* 1986	100	300	245[e]	203[e]

N.D. not determined
[a]Maximum likelihood estimate for BMR = 0.1 and $P_0 = 0.05$ for continuous endpoints.
[b]Lower bound estimate for BMR = 0.1 and $P_0 = 0.05$ for continuous endpoints.
[c]Results for Weibull and Power models. Unadjusted for continuous exposure
[d]Results for Weibull and Power models.
[e]Results for Power model.

Kidney Toxicity:

Kidney toxicity was reported in rats at the two higher exposure concentrations, and a NOAEL was found at the lowest concentration of 100 ppm. The NOAEL was adjusted to 21 ppm for the 7 h/day, 5 day/week exposure and divided by a total UF of 30 (10 for H and 3 for A) giving the RfC of 0.7 ppm (see Table 12).

Exposure Dose-Based BMDs and RfCs

Neurological Effects:

Four continuous measures (electroencephalographic and electrocardiographic) were reported in this study and the MLEs and BMDLs are reported in Table 7. The BMDLs vary by as much as 16-fold using the two models (Weibull and Power) (Figures 5 a, b). One option to improve the fit of the models would be to drop the highest dose because we are interested in the BMDs at lower concentrations where the response is most linear. The BMDLs were used to calculate the RfCs by adjusting for the intermittent exposure (8 h/d, 5 d/week) and using a total UF of 30 (10 for H and 3 for A). The RfC for the Weibull and Power models, respectively, are: heart rate - 0.3 and 1 ppm, wakefulness - 0.06 and 1 ppm, slow-wave sleep - 0.06 and 0.6 ppm, and paradoxical sleep - 2 and 0.9 ppm for Weibull and Power models, respectively (Tables 8 and 12).

Liver to Body Weight Ratio:

In contrast to the identical LOAELs for female and male mice, the BMDs based upon exposure dose are different due to the different shapes of the dose response. The females show a basically linear increase with dose in LW/BW, while the male dose-response is concave. Estimates of MLE were 44 and 27 ppm for females using the Weibull and Power models, respectively, while estimates of BMDL were 32 and 21 ppm using the two models. For males, the MLE estimates were 11 and 30 ppm while the BMDL estimates were 5 and 24 ppm. The BMDLs were divided by a total UF of 30 (10 for H and 3 for A) to obtain RfCs of 1 and 0.7 ppm from female mouse data and 0.2 and 0.8 ppm from male mouse data using the Weibull and Power models, respectively (see Table 12).

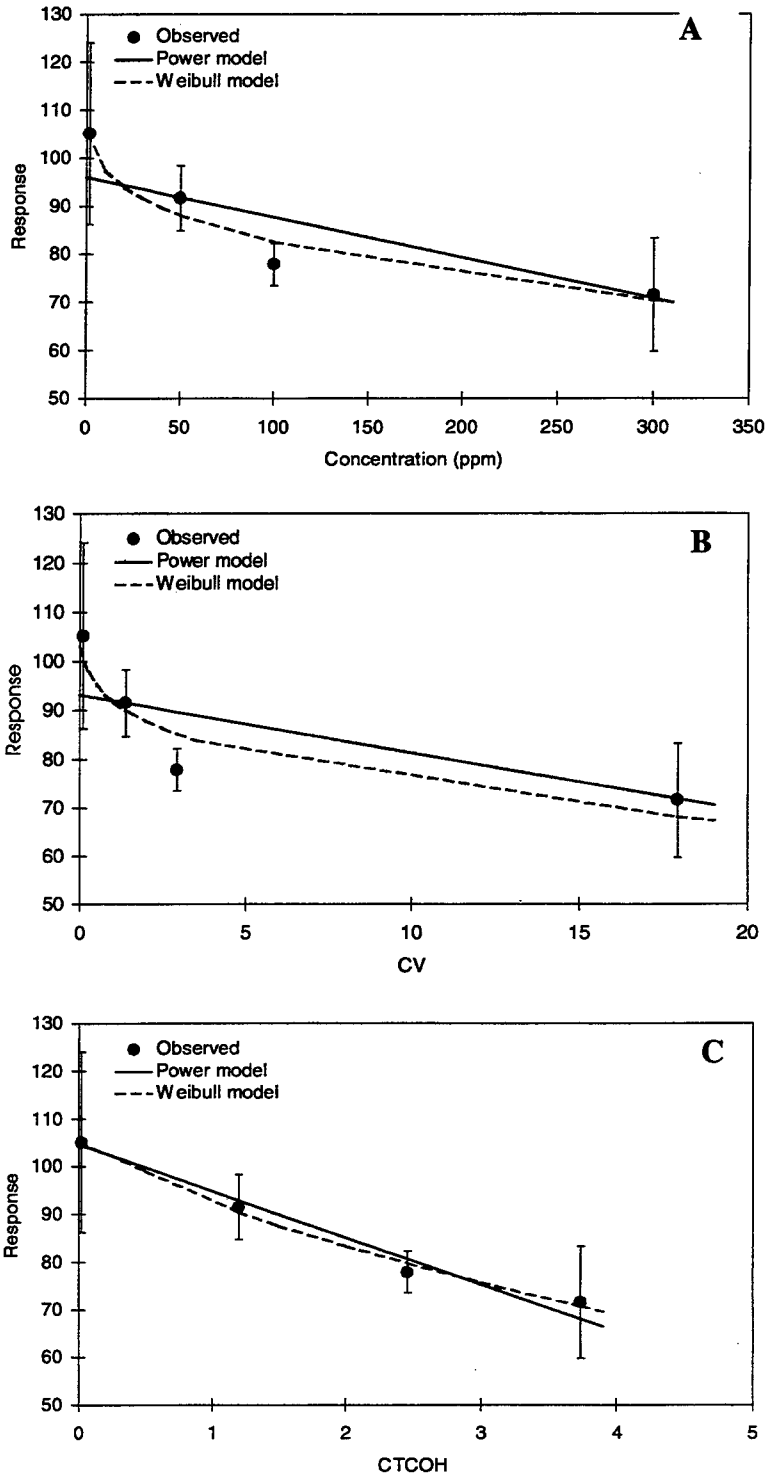

Figure 5: Neurological effects. Model fits to wakefulness electroencephalographic data using alternate dose metrics: (A) exposure concentrations (P_0=0.01, BMR=0.1), (B) venous TCE (CVTCE) (P_0=0.01, BMR=0.1), (C) blood TCOH concentrations (CTOCOH) (P_0=0.01, BMR=0.1).

TABLE 8: BMDS AND RfCS FOR NEUROLOGICAL EFFECTS (BMR=0.1, P_0=0.1) (ARITO *ET AL.* 1994)

Model	MLE	BMDL	RfC-MLE	RfC-BMDL
HR[1], *exposure*			*30*	*1*
Weibull, P_0	124	32	1	0.3
K Power, P_0	279	122	2	1
W[2], *exposure*			*30*	*1*
Weibull, P_0	23	8	.2	0.06
K Power, P_0	253	130	20	1
SWS[3], *exposure*			*30*	*1*
Weibull, P_0	32	8	0.3	0.06
K Power, P_0	161	78	1	0.6
PS[4], *exposure*			*30*	*1*
Weibull, P_0	6478	223	51	2
K Power, P_0	373	115	3	0.9
HR, AUCTCE			*10*	*1.8*
Weibull, P_0	58	15	10	3
K Power, P_0	155	64	28	11
HR, AUCTCH			*10*	*0.3*
Weibull, P_0	20	4.7	1	0.2
K Power, P_0	29	14	1	0.5
HR, CVTCE			*10*	*52*
Weibull, P_0	8	2	40	10
K Power, P_0	21	8	108	44
HR, CTCOH			*10*	*8*
Weibull, P_0	2	0.6	2	0.4
K Power, P_0	3	1	2	1
W, AUCTCE			*10*	*1.8*
Weibull, P_0	10	4	2	0.7
K Power, P_0	144	74	26	13
W, AUCTCH			*10*	*0.319*
Weibull, P_0	6	1	0.2.	0.04
K Power, P_0	23	12	0.7.	0.4

[1] HR = heart rate
[2] W = wakefulness
[3] SWS = slow wave sleep
[4] PS = paradoxical sleep

Table 8 (cont.)

Model	MLE	BMDL	RfC-MLE	RfC-BMDL
W, CVTCE			*10*	*52*
Weibull, P_0	1	0.5	7	3
K Power, P_0	19	10	100	51
W, CTCOH			*10*	*8*
Weibull, P_0	0.9.	0.2	0.7	0.1
K Power, P_0	2	1	2	0.8
SWS, AUCTCE			*10*	*2*
Weibull, P_0	10	4	2	0.7
K Power, P_0	96	45	17	8
SWS, AUCTCH			*10*	*0.3*
Weibull, P_0	8	2	0. 3	0.06
K Power, P_0	15	8	0.3	0.2
SWS, CVTCE			*10*	*52*
Weibull, P_0	1	0.5	7	3
K Power, P_0	13	6	67	32
SWS, CTCOH			*10*	*8*
Weibull, P_0	1	0.4	.9	0.3
K Power, P_0	1	0.8	1	0.6
PS, AUCTCE			*10*	*1.8*
Weibull, P_0	7028	126	1246	22
K Power, P_0	203	50	36	9
PS, AUCTCH			*10*	*0.3*
Weibull, P_0	345	26	11	0.8
K Power, P_0	43	16	1	0.5
PS, CVTCE			*10*	*52*
Weibull, P_0	896	18	4656	92
K Power, P_0	28	6	143	33
PS, CTCOH			*10*	*8*
Weibull, P_0	18	3	14	2
K Power, P_0	4	2	3	1

[1] HR = heart rate
[2] W = wakefulness
[3] SWS = slow wave sleep
[4] PS = paradoxical sleep

Kidney Toxicity:

For the inhalation data sets, the Polynomial and Weibull models gave very similar results (Figure 6). To be consistent with the oral data, the results from fitting the Polynomial model were used here. The MLE was 245 ppm and the BMDL was 203 ppm. The BMDL was multiplied by 7/24 and 5/7 to adjust to continuous dosing and then divided by a total UF of 30 (10 for H and 3 for A) to obtain the RfC of 1 ppm (see table 12).

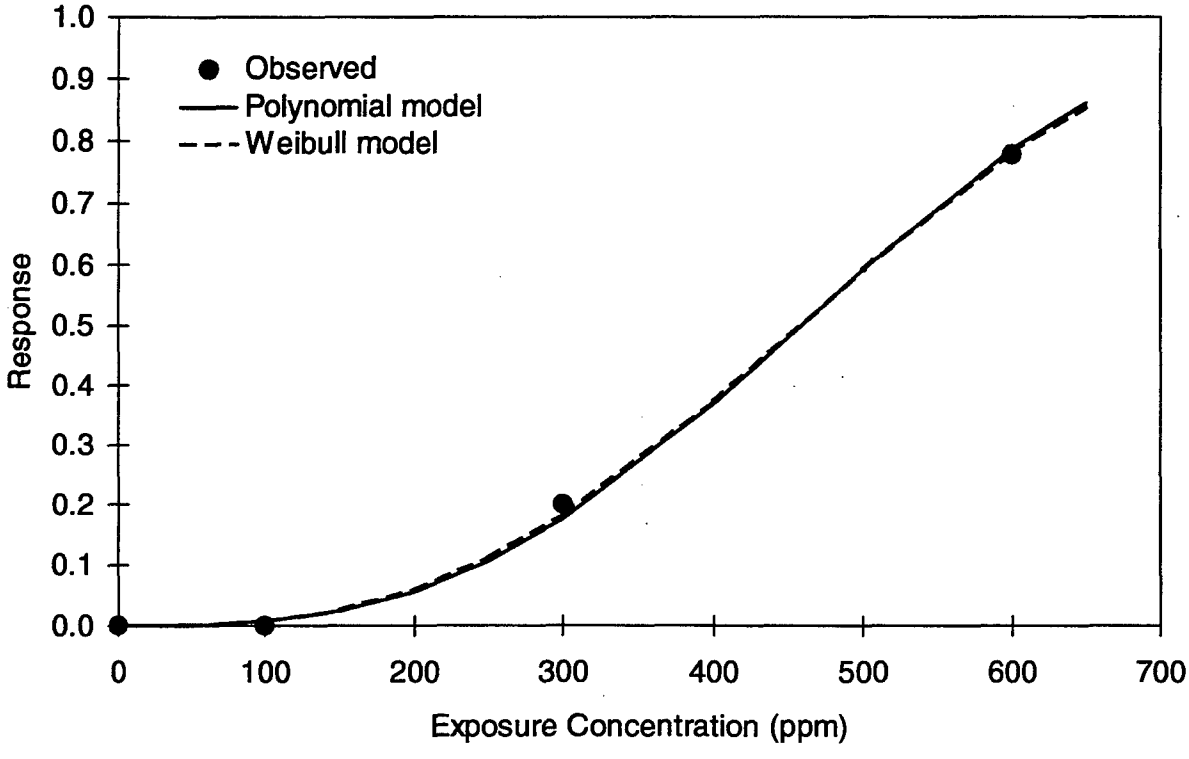

Figure 6: Kidney effects. Model fits using inhalation exposures (BMR=0.1)(Maltoni et al. 1986).

INHALATION STUDIES - INCORPORATING PHARMACOKINETICS AND PHARMACODYNAMICS

Studies to be evaluated as potential critical studies were simulated with the PBPK model to obtain estimated internal dose metrics. The values for the dose metrics at the NOAELs or LOAELs were determined. In addition, these values were used to calculate the BMDs expressed

as a function of the internal dose metrics. In order to utilize a dose metric, the relationship of the metric to the effect must be described, i.e. pharmacodynamics. As with incorporating pharmacokinetics into dose-response assessment, this may be done in several ways: utilizing data, using physiological-based models, or using default assumptions. Whichever approach is used, information is needed for both the animal and humans in order to extrapolate to calculate human exposure doses.

Selection of Pharmacokinetic Dose Metrics and Pharmacodynamic Adjustments

Neurological Effects:

There are two major hypotheses for the mode of action of TCE in the causation of neurological effects (Blain *et al.* 1994). One possibility is that parent TCE is responsible, while the other focuses on TCOH. Another active species may be chloral hydrate, but it is generally believed that TCOH is the most significant active species in choral hydrate anesthesia because it is relatively long-lived while choral hydrate is very rapidly metabolized to TCOH (Breimer 1977). In the absence of more detailed information or hypotheses about the mode of action, either AUC or peak concentrations might be a reasonable dose metric. Therefore, four possibilities were evaluated: AUCTCE, AUCTCH, CVTCE, and CTCOH. These dose metrics were obtained by estimating with the model the daily AUC or concentration during the five exposure days of the sixth week of the experiment.

Although data or models were not available describing the pharmacodynamic process in animals, there was some comparable data in humans to the neurological data in the rodent studies. These data provided a basis for comparing the responsiveness of the two species. One limitation of the human data was that all the studies use exposures lasting one day or, in one study, five days. The importance of this is somewhat unclear because Arito *et al.* (1994) report some effects show a dependence upon repeated exposures while other do not. In addition, some effects were dependent upon repeated exposures but not on exposure concentration. The dose metrics at the LOAEL dose of 50 ppm were compared with the dose metrics in the human studies (Table 9).

As shown in Table 9, studies using 27 to 200 ppm report slight neurological effects. Heart rate was reported in three studies to either be unaffected or to show a slightly decreasing trend. The internal dose metrics modeled for the human studies are generally equal to or greater than those in the rat study, particularly for TCOH. Exceptions occur in the dose metrics for TCE. Due to the slower clearance of TCOH in humans arising from enterohepatic recycling, the modeled peak concentration and AUCTCH is consistently higher than those found for the rat.

TABLE 9: COMPARISON OF DOSIMETRICS FROM RAT AND HUMAN NEUROLOGICAL STUDIES

Study	Exposure Concen-tration (ppm)	Effects	CVTCE	CTCOH	AUCTCE	AUCTCH
Arito *et al.* 1994	50 for 8 h per day LOAEL	Electroencephalographic changes in sleep and wakefulness; decreased heart rate	1.3	1.2	13	10
Human Studies						
Stewart *et al.* 1970	200 for 7 h for 5 days	Mild fatigue and sleepiness on days 4 and 5	3.8	15.5	34	206
Nomiyama and Nomiyama 1977	27 for 4 h 81 for 4 h	Slight trend toward slower pulse rate	0.4 1.3	1.4 4.2	2.4 7.4	14 43
Windemuller and Ettema 1978	200 for 2.5 h	No effect on heart or breathing rate	3.0	6.5	12	66
Salvini *et al.* 1971	110 for 8 h	Decreased performance on psychophysiological tests	1.9	9.8	20	127
Konietzko *et al.* 1975	95 for 4 h	No effect on heart rate	1.5	4.9	8.7	51

These data do not support the default assumption that humans are more sensitive than the rat. The studies show little or no effect in humans with modeled internal dose metrics equal to or greater than those modeled for the rat exposed at a concentration producing minimal effects.

Therefore, the UF for interspecies extrapolation was given a value of 1 when applied to internal dose metrics.

Liver to Body Weight Ratio:

Possible dose metrics for this effect were discussed above (Section 3.5.1) and for consistency AUCTCA was evaluated for inhalation also. The AUCTCA was obtained by estimating with the model the daily average AUCTCA during the 30-day continuous exposure. As described previously for oral exposures, the data do not support the default assumption that humans are more sensitive than animals for liver effects, so a value of 1 was used for the interspecies extrapolation based upon internal dose metrics. A 10-fold adjustment for H was also made although, as previously discussed, the mice may essentially be equivalent to a sensitive subpopulation with a fully active PPAR that has not yet been identified in humans.

Kidney Toxicity:

The dose metric, KTOX, was evaluated for inhalation exposures as it was for oral exposures. The average daily value for KTOX was obtained by averaging over a one-week (7-day) period. The default assumption that humans are 3-fold more sensitive than animals was used with internal dose metrics due to a lack of data.

Dose Metric-Based NOAELs and RfCs

Neurological Effects:

The exposure LOAEL of 50 ppm resulted in the following values for potential internal dose metrics: AUCTCE 13 mg*hr/L, AUCTCH 10 mg*hr/L, CVTCE 1.4 mg/L, and CTCOH 1.2 mg/L. These are peak concentrations or average daily values for AUCs, so no further dose adjustments were needed for the intermittent exposure. These dose metrics were divided by a total UF of 30 (3 for L, 10 for H and 1 for A) and by the appropriate value from Table 4 to convert to an equivalent human exposure concentration. The RfCs obtained were 0.8, 0.1, 2, 0.3 ppm based upon AUCTCE, AUCTCH, CVTCE, and CTCOH, respectively.

Liver to Body Weight Ratio:

The exposure NOAEL of 37 ppm resulted in a value for AUCTCA of 2789 mg*h/L. The AUCTCA was divided by a total UF of 10 (3 for L, 10 for H and 1 for A) and by 243 to obtain the equivalent human exposure concentration giving the RfC of 0.4 ppm.

Kidney Toxicity:

The value of KTOX for the lowest concentration exposure, the NOAEL, was 39 mg/L. KTOX was divided by a total UF of 30 (10 for H and 3 for A) and by 0.74 to obtain the equivalent human exposure concentration giving the RfC of 2 ppm.

Dose Metric-Based BMDs and RfCs

Neurological Effects:

The BMDs obtained using the dose metrics are reported in Table 8. As illustrated in Figure 5c, the peak concentration of TCOH linearized the data, though the AUCTCH tended to be an improvement over exposure concentrations or blood levels of TCE. This suggests that TCOH is the active agent although involvement of the parent, TCE, cannot be ruled out. Each BMDL was divided by a total UF of 10 (10 for H and 1 for A) and by the appropriate value to obtain the equivalent human exposure concentrations. The RfCs ranged from 0.04 to 99 ppm for all endpoints and the four dose metrics: heart rate - 0.2 to 44 ppm, wakefulness - 0.04 to 51 ppm, slow-wave sleep - 0.06 - 32 ppm, and paradoxical sleep - 0.5 - 92 ppm. Several factors contributed to this variability. Values based upon TCE were consistently higher than those based upon TCOH due to the slow clearance of the latter in humans. The highest numbers arose from CVTCE, generally from the Power model; values with the Weibull were lower by as much as 10-fold except in the case of paradoxical sleep. The lowest values were generally obtained from the Weibull model fit using AUCTCH and the values with the Power model were up to 10-fold higher, except again for paradoxical sleep. The most linear results were obtained using CTCOH, so the values based upon the two models were the most similar with this dose metric. Averaging the results obtained from the two models for each endpoint, the most health protective RfC would be 0.4 ppm based upon slow-wave sleep.

Liver to Body Weight Ratio:

The values of the MLE and BMDL using AUCTCA for the dose surrogate were much less variable than when based upon the exposure concentrations, particularly for males, indicating better fits of the models (Table 10). The BMDLs for female mice were divided by a total UF of 10 (10 for H and 1 for A) and by 231 to obtain the equivalent human exposure concentration giving an RfC of 1 and 0.9 ppm for the Weibull and Power models, respectively. The RfCs based upon the male mice were calculated identically giving 0.5 ppm for both models.

TABLE 10: BMDS AND RfCS FOR LW/BW BASED UPON INTERNAL DOSE METRICS (KJELLSTRAND *ET AL.* 1983A)

	MLE-AUCTCA (mg*h/L)	BMDL-AUCTCA (mg*h/L)	RfC-MLE[a] (ppm)	RfC-BMDL[a] (ppm)
Female mice				
Weibull	2851	2496	1	1
Power	2382	2067	1	0.9
Male mice				
Weibull	1635	1214	0.7	0.5
Power	1538	1122	0.7	0.5

[a]RfCs based upon UF = 10 and dividing by 232 to convert AUCTCA to human equivalent exposure dose

Kidney Toxicity:

The values of KTOX using the Polynomial model are 623 mg/L and 370 mg/L for the MLE and BMDL, respectively. The maximum likelihood estimates for KTOX for the identical response (i.e. BMR = 0.1) are very similar for the oral and inhalation routes, 673 and 623, demonstrating good route-to-route extrapolation using the model. The BMDL was divided by a total UF of 30 (10 for H and 3 for A) and by 0.74 to obtain the equivalent human exposure concentration giving an RfC of 17 ppm.

DISCUSSION AND SUMMARY

The process of developing dose-response values is an iterative one, particularly when utilizing pharmacokinetic and mode of action information. The initial step is to review the literature and chose the studies that might be used as critical studies for each toxic endpoint. The supporting literature needs to be reviewed to determine the availability of hypotheses for the mode of action leading to the various effects. The NOAEL or BMDs can be determined using the exposure doses or internal dose metrics. Pharmacokinetic analyses can then be undertaken to obtain internal tissue dose metrics in the animals used in the studies. Next the mode of action must be evaluated to determine the relationship of the internal dose metric to the effect and the proper extrapolation to humans. These results are then extrapolated to humans using both the pharmacokinetic and mode of action information. Finally, the various possible RfDs and RfCs must be compared with each other and with any other relevant literature to evaluate if any other factors need to be considered. Overall, RfDs and RfCs developed using the BMD method, pharmacokinetic analysis, and mode of action considerations appear the most desirable because they incorporate the greatest amount of the scientific database into the regulatory process.

Oral Studies

The values obtained for RfDs derived using the NOAEL/LOAEL, BMDL, PK-NOAEL/LOAEL, and PK-BMDL are listed in Table 11. The lowest RfDs were derived for the liver effects and eye malformations. Kidney toxicity and immunotoxicity gave the highest RfDs. The rank ordering varies depending upon the method used.

Eye Malformations

Two factors strongly affected the derivations of the RfDs for this endpoint, use of the BMD method and the choice of an internal dose metric. The LOAEL reported in Narotsky *et al.* (1995) based upon a comparison of treatment groups and controls was 1125 mg/kg/d, while the trend test gave a LOAEL of 101 mg/kg/d (Barton and Das 1996). At the LOAEL, 4.4% of the pups

TABLE 11: SUMMARY OF RFDS FOR ALL ENDPOINTS

Model	NOAEL / BMD	Exposure Adjustments	UF*	Basis for UF	Dose metric adjustment	RfD[d] (mg/kg/d)
Eye Malformations – Narotsky *et al.*, 1995						
NOAEL	32[a]		100	H:10, A:10	1	0.3
BMDL	500[a]		100	H:10, A:10	1	5
PK-NOAEL: TCA	71[b]		30	H:10, A:3	479	0.005
PK-BMDL: TCA	2924[b]		30	H:10, A:3	479	0.2
LW/BW -- Tucker *et al.* 1982						
NOAEL	18[a]		100	H:10, A:10	1	0.2
PK-LOAEL	570[b]		30	H:10, A:3	479	0.04
LW/BW – Buben and O'Flaherty, 1985						
LOAEL	100[a]	5/7	300	H:10, A:10, L:3	1	0.2
BMDL -Weibull	14[a]	5/7	100	H:10, A:10	1	0.1
BMDL - Power	341[a]	5/7	100	H:10, A:10	1	2
PK-LOAEL	573[b]		30	H:10, A:1,	479	0.04
PK-BMDL - Weibull	551[b]		10	H:10, A:1	479	0.12
PK-BMDL - Power	196[b]		10	H:10, A:1	479	0.04
LW/BW – Berman *et al.*, 1995						
LOAEL	50[a]		300	H:10, A:10, L:3	1	0.2
BMDL -Weibull	742[a]		100	H:10, A:10	1	7
BMDL - Power	650[a]		100	H:10, A:10	1	7
PK-LOAEL	118[b]		30	H:10, A:1, L:3	479	0.008
PK-BMDL - Weibull	308[b]		10	H:10, A:1	479	0.06
PK-BMDL - Power	239[b]		10	H:10, A:1	479	0.05
Immunotoxicity – Sanders *et al.*, 1982						
NOAEL	200[a]		100	H:10, A:10	1	2
Kidney Toxicity – Maltoni *et al.*, 1986						
NOAEL	50[a]	5/7	100	H:10, A:10	1	0.4
BMDL	70[a]	5/7	100	H:10, A:10	1	0.5
PK-NOAEL	85[c]		30	H:10, A:3	1.54	2
PK-BMDL	233[c]		30	H:10, A:3	1.54	5

* UF = Uncertainty Factors

[a] mg/kg/day exposure dose metric

[b] mg*hr/L internal dose metric – AUCTCA

[c] mg/kg internal dose metric – KTOX

[d] RfD is obtained by multiplying NOAEL/BMD by adjustment for exposure and dividing by UF and dose metric adjustment.

were affected and as shown in Figure 2, the dose-response rises slowly. Therefore, use of the BMD method and the choice of the BMR affect the RfD. The BMDL was 500 mg/kg/d for a BMR of 0.1 and 240 mg/kg/d for a BMR of 0.05. Analysis of a large database of developmental studies found the BMR = 0.05 gave results that on average were most like the NOAEL, when litter data were available (Allen *et al.*1995). The RfDs would be 5 and 2 mg/kg/d based upon the choice of BMR. Using AUCTCA as the dose metric, the RfDs would be 0.2 or 0.1 based upon the choice of BMR.

The choice of the internal dose metric was also important. The range of RfDs obtained using several possible dose metrics at the NOAEL was 0.005 to 2 mg/kg/d. The lowest value was obtained for the AUCTCA due to the slow clearance of TCA in humans compared to rodents. Data with TCA dosing supported that it caused effects similar to those seen with TCE. If TCA is the active agent, something is not captured in the dose metric chosen because the AUCTCA causing the response was much higher for dosing with TCA than with TCE. The PK-BMD analysis was done using AUCTCA because it was the most conservative and because TCA dosing can cause a similar effect. The RfD obtained for BMR = 0.1 was 0.2 mg/kg/d, while that obtained for BMR=0.05 was 0.1 mg/kg/d.

Liver Toxicity

Once again the choices of using the BMD method and selection of AUCTCA as the internal dose metric affected the RfDs, although the two factors worked in opposite directions so that values obtained using the PK-BMD were not that different from those derived using the LOAEL. In addition, the results from the three studies were fairly similar. The RfDs based upon the PK-BMDL were 0.08 and 0.06 mg/kg/d (averaging the results from the two models) which compares well with the value of 0.04 mg/kg/d obtained from the PK-NOAEL for Tucker *et al.* (1982). The major question regarding the liver toxicity is the interspecies extrapolation of the mode of action. We have included LW/BW because it appears to be a useful indicator of pleiotropic changes associated with the PPAR. In mice, these changes include peroxisome proliferation and the eventual development of tumors through a negative selection process (Andersen *et al.* 1995). Peroxisome proliferation occurs less readily in rats and much less readily or not at all in humans.

This was the basis for selecting a value of 1 for A, but it is possible the value should be fractional.

Immunotoxicity

The least information was available for immunotoxicity, so only a NOAEL was used to develop an RfD of 2 mg/kg/d. Any or all of the dose metrics considered for other endpoints could have been evaluated. Those for stable metabolites would lead to lower RfDs if it were also assumed that humans were pharmacodynamically equally or more sensitive than the rodents.

Kidney Toxicity

The RfDs for kidney toxicity were dependent upon the use of pharmacokinetic modeling. Incorporating pharmacokinetics raised both the NOAEL and BMDL-based values despite the assumption that humans are pharmacodynamically more sensitive than rodents. This increase is due to the greater detoxification of the GSH pathway by N-acetylation in humans as compared to rodents. However, there is limited information and a degree of uncertainty regarding the quantitation of the GSH pathway (see Clewell *et al.* 1998).

Inhalation Studies

The values obtained for RfCs derived using the NOAEL/LOAEL, BMDL, PK-NOAEL/LOAEL, and PK-BMDL are listed in Table 12. The lowest RfCs were derived for the liver and neurological effects. Kidney toxicity gave the highest RfCs. The rank ordering varies depending upon the method used.

Neurological Effects

While the LOAEL was 50 ppm for all four endpoints, the BMD analysis varied. Wakefulness and slow-wave sleep were inversely correlated with electroencephalographic measurements, while paradoxical sleep and heart rate were relatively independent measurements. The BMDL-based RfCs varied from 0.06 to 2 ppm and tended to vary greatly with the two BMD models used.

TABLE 12: SUMMARY OF RFCS FOR ALL ENDPOINTS

Model		NOAEL / BMD	Exposure Adjustments	UF*	Basis of UF	DMA$^{\perp}$	RfC (ppm)
Neurological Effects – Arito *et al.*, 1994							
LOAEL		50	(8/24)(5/7)	100	H:10, A:3, L:3		0.1
BMDLs							
HR	Weibull	32	(8/24)(5/7)	30	H:10, A:3	1	0.3
HR	Power	122	(8/24)(5/7)	30	H:10, A:3	1	1.0
W	Weibull	8	(8/24)(5/7)	30	H:10, A:3	1	0.06
W	Power	130	(8/24)(5/7)	30	H:10, A:3	1	1.0
SWS	Weibull	8	(8/24)(5/7)	30	H:10, A:3	1	0.06
SWS	Power	78	(8/24)(5/7)	30	H:10, A:3	1	0.6
PS	Weibull	223	(8/24)(5/7)	30	H:10, A:3	1	2
PS	Power	114	(8/24)(5/7)	30	H:10, A:3	1	0.9
PK-LOAELs: CTCOH		1.2		30	H:10, A:1, L:3	0.132	0.3
PK-BMDLs: CTCOH							
HR		0.99		10	H:10, A:1	0.132	0.8
W		0.64		10	H:10, A:1	0.132	0.5
SWS		0.57		10	H:10, A:1	0.132	0.4
PS		2.2		10	H:10, A:1	0.132	1.7
LW/BW – Kjellstrand *et al.*, 1983a							
LOAEL		37		100	H:10, A:3, L:3	1	0.4
BMDL							
female	Weibull	32		30	H:10, A:3	1	1
female	Power	21		30	H:10, A:3	1	0.7
male	Weibull	5		30	H:10, A:3	1	0.2
male	Power	24		30	H:10, A:3	1	0.8
PK-LOAEL		2789		30	H:10, A:1, L:3	232	0.4
PK-BMDL							
female	Weibull	2496		10	H:10, A:1	232	1
female	Power	2067		10	H:10, A:1	232	0.9
male	Weibull	1214		10	H:10, A:1	232	0.5
male	Power	1122		10	H:10, A:1	232	0.5
Kidney Toxicity – Maltoni *et al.*, 1986							
NOAEL		100	(7/24)(5/70	30	H:10, A:3	1	0.7
BMDL		203	(7/24)(5/7)	30	H:10, A:3	1	1
PK-NOAEL		39		30	H:10, A:3	0.74	2
PK-BMDL		370		30	H:10, A:3	0.74	17

* Uncertainty Factor

$^{\perp}$ Dose Metric Adjustment

The pharmacokinetic analysis was particularly interesting because it suggests that TCOH was the active form, rather than TCE. The best linearization of the data for all four endpoints was obtained using the peak concentration of TCOH. An important consideration is whether humans are more sensitive than animals. Though the data are limited to single exposures, the data is not supportive of greater sensitivity. Therefore, it was assumed that humans and rodents are equally sensitive to TCOH.

Another issue for this study is what adjustments are appropriate for the intermittent animal exposure and less than chronic duration. The study provided inconsistent evidence of small changes with repeat exposure for the different endpoints measured during and following the exposure period. The 6-week studies were used in this analysis because these tended to be the most dose-dependent. It is particularly difficult to understand the apparent dependence of some measurements on repeated exposure but not on dose. Due to this limited evidence for dependence on exposure duration and the utility of CTOH as the internal dose metric, the uncertainty factor for less than chronic exposure was not used (or valued at 1), but adjustments were made from intermittent to continuous exposure assuming the concentration-time product was constant. These adjustments reduced the exposure concentration by 4.2 ($24/8 \times 7/5$).

The BMDLs tended to be dependent upon the model used even when CTOH was used as the dose metric. There does not appear to be any basis upon which to prefer one model over the other, so the results were averaged. RfCs based upon CTOH and PK-BMDLs for slow-wave sleep and wakefulness were very similar, 0.4 and 0.5 ppm, respectively. It should also be noted that when using CTOH as the dose metric, there was little or no effect of pharmacokinetics and mode of action as compared to RfCs based upon exposure concentrations. By comparison, dose metrics based upon TCE would raise the RfCs.

Liver Effects

The increases in LW/BW were evaluated separately for female and male mice, though the LOAELs were identical (37 ppm). While the BMDs were somewhat model dependent when based upon exposure doses, they were very similar when based upon AUCTCA. The RfC based

upon males was half that based upon females using the PK-BMDLs, 0.5 and 1 ppm, respectively. The issues previously discussed for liver effects following oral exposure are also applicable to the inhalation data because the endpoint and mode of action are the same.

Kidney Toxicity

As for oral exposures, the RfCs based upon kidney toxicity tended to be higher than those for the other endpoints. This was especially true for the RfC based upon the PK-BMDL. While there is perhaps more uncertainty associated with the parameter values for humans for the GSH pathway, the appropriateness of KTOX as a dose metric was supported by the its similarity with both the inhalation and oral routes of exposure. Using the polynomial model and BMR = 0.05, the MLE estimate was 548 mg/L by oral exposure and 356 mg/L by inhalation.

Final Considerations

This analysis used oral studies to develop RfDs and inhalation studies to develop RfCs, but it would also be possible using PBPK modeling to do route-to-route extrapolations for endpoints that were available by only one exposure route. Consideration should be given to using this approach to develop an RfD for neurological effects and an RfC for developmental effects using the oral eye malformation data. A similar extrapolation of the oral immunotoxicity results to inhalation would be possible. The critical factor for these extrapolations is the selection of the appropriate dose metric, which is particularly problematic for the immunotoxicity data and to a lesser extent for the oral developmental data.

It is also important to consider interchemical comparisons when developing RfD/Cs. Heavy use has been made in this analysis of information for metabolites of TCE and some comparisons were made to TCA production from perchloroethylene. Therefore, if RfD/Cs are developed for these other chemicals, their relationship to the values for TCE needs to be considered. It would, for instance, probably be inappropriate, to develop an RfD for TCA that was so low that more TCA was allowed following TCE exposure. Currently no values are available for these other chemicals.

Several studies were not selected as potential critical studies, but warrant consideration at this point. These include both inhalation immunotoxicity and oral developmental studies.

The inhalation studies of decreased lung macrophage function were not selected because of the brief exposure durations used which make extrapolation to chronic RfCs difficult (Aranyi *et al.* 1986, Park *et al.* 1993). At the lowest exposure concentration used, 2.5 ppm, there was a small increase in response following 5 daily exposures but not a single exposure. The BMDL based upon exposure concentrations and a BMR = 0.10 would be 7 ppm. The RfCs based upon neurological and liver effects provide varying, but small margins of exposure relative to this acute effect depending upon their derivations. The RfCs for kidney toxicity similarly provide small margins of exposure except when based upon the PK-BMDL. Therefore, the use of the lowest RfC in risk assessments intended to be protective for all noncancer effects will provide some margin of exposure for this effect.

Following oral dosing, it has been reported that there were neurological effects, both behavioral and biochemical, and an increased incidence of cardiac malformations. The neurological studies used exposures during pregnancy and for 21 days after birth (Taylor *et al.* 1985, Isaacson and Taylor 1989). Limited data is provided to estimate maternal exposure doses (approximately 16 and 32 mg/kg/d), but additional information would be required to estimate the postpartum exposure of the pups. The RfDs developed for other endpoints would all provide a margin of exposure protective from these effects, although those based upon kidney toxicity would provide a relatively small one.

The study reporting cardiac malformations was considered problematic due to the lack of dose-response when considering total malformations and the limited dependence upon time of exposure when considering either total malformations or atrial septal defects. The doses were estimated to be approximately 0.2 and 84 mg/kg/d and responses above background varied from about 3 to 8%. As was seen with the eye malformations, which also had a very shallow dose response relationship, the MLE and BMDL may be expected to be towards the higher value. Previous efforts to do such analyses have had considerable difficulty because the mathematical models were unable to

satisfactorily fit both data points (unpublished data). All the RfDs derived for other endpoints provide margins of safety, some quite large, relative to the higher dose. Depending upon the method used in their derivations, the RfDs range from somewhat below the lower dose to much greater. The RfDs based upon immunotoxicity and kidney toxicity would not provide any margin of exposure relative to the lower dose.

If there were concern that the margins of exposure were not great enough for any of these effects, consideration should be given to using a value of 3 or 10 for the UF for database. While this value is typically used when there is a lack of data, it would appear there is also uncertainty in the database when there are limited positive findings. This option follows from a proposal for addressing comparative risks of chemicals, that regulatory values fully utilize data that is generally agreed to and then provide partial protectiveness in areas of some disagreement (Garetz 1993). The use of this UF would clearly be dependent upon all the choices made in deriving the RfDs and RfCs, particularly the selection of BMR, P_0, dose metrics, and pharmacodynamic extrapolations. If these were to change, this final comparison would need to be redone because it represents policy choice.

The process of developing dose-response values is an iterative one that typically is repeated, as additional scientific information becomes available. The exposure : dosimetry : mode-of-action : response framework described in this paper organizes the process and promotes consistency between endpoints. This framework facilitates incorporating scientific data and assists scientists conducting research by defining methods by which scientific data can be used in risk assessment. These methods will include: BMDs derived by empirical curve fitting, pharmacokinetic models reflecting the processes important for different chemicals and their metabolites, use of uncertainty factors to semi-quantitatively adjust for incompletely described pharmacokinetic and pharmacodynamic processes, and eventually more complete pharmacodynamic models that quantitatively describe critical steps in the mode of action leading to toxicity. The choice of methods used will vary in response to the availability of data as well as differences in the relevant biological processes. Appropriate dose-response analysis requires a consistent framework for organizing information and analyses, not a single universally applied analytical method.

The initial step in the process is to review the literature and choose the studies that might be used as critical studies for each toxic endpoint. The supporting literature needs to be reviewed to determine the availability of hypotheses for the mode of action leading to the various effects. The NOAELs or BMDs can be determined using exposure doses or internal dose metrics; absent mode of action and pharmacokinetic information, analyses based upon exposure dose represent an appropriate default approach. When feasible, pharmacokinetic analyses should be undertaken to obtain internal tissue dose metrics in the animals used in the studies. Next the mode of action must be evaluated to determine the relationship of the internal dose metric to the effect and the proper extrapolation to humans. These results are then extrapolated to humans using both dosimetry and mode of action information. Finally, the various possible RfDs and RfCs must be compared with each other, as well as any other relevant literature, to determine if any other factors need to be considered. Overall, RfDs and RfCs developed using the BMD method with pharmacokinetic dosimetry and consideration of mode of action appear the most desirable because they incorporate the greatest amount of the scientific database into the regulatory process.

One measure of the utility of this approach is the consistency obtained for systemic effects regardless of whether the exposure was oral or by inhalation. The BMDs and toxicity values derived from them (i.e. RfDs and RfCs) for both liver and kidney effects are very similar by these two routes. For example, the maximum likelihood estimates for the kidney dose metric (KTOX) at the same response level (BMR = 0.1) are very similar for the oral and inhalation routes, 673 and 623 mg/L respectively, demonstrating good dose-route extrapolation using the model. The target tissue metrics (AUCTCA) underlying the RfC and RfD for liver effects are also similar, differing by a factor of only 2 – 3, though based upon studies using different rodent strains, durations, and routes of exposure.

The analyses presented here have included endpoints with widely varying databases. Effects included those for which virtually no data other than exposure and response were available (i.e. eye malformations) and others with varying amounts of data on mode of action and pharmacokinetics that inform extrapolations between exposure regimens (e.g. less than chronic to chronic liver

effects) or between species (e.g. neurological effects). Overall, the analyses suggest that an RfD in the range of 0.06 to 0.12 mg/kg/day based upon liver effects analyzed with AUCTCA as the internal dose metric would also be protective for the other endpoints evaluated. Similarly, an RfC in the range of 0.4 to 1.0 ppm based upon slow-wave sleep analyzed with CTCOH as the internal dose metric would be protective for the other endpoints, as well. Modifications of these numbers might arise from the use of alternative approaches for deriving the BMDs or from different interpretations of the mode of action and pharmacokinetic considerations informing selection of uncertainty factor values.

One of the significant benefits of attempting to consider a broad range of toxicity endpoints as well as pharmacokinetic and mode of action information is that it makes more apparent where there are limitations in the database. While there are far too many studies reporting alterations in LW/BW, additional studies of the mode of action leading to liver toxicity could be useful. Developmental studies in one species of rat that attempted to resolve the apparent discrepancy between the ability of TCA to induced both eye and cardiac malformations but at internal concentrations that would not be pharmacokinetically consistent with its being the active agent from TCE would be useful. Studies that follow-up on pulmonary macrophage activity or brain demyelination might also be appropriate particularly if they provided a pharmacokinetic and pharmacodynamic context. Finally, additional data for DCA would be useful, for better understanding interspecies comparisons of its pharmacokinetics and mode of action.

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APPENDIX A

TABLES OF TABLES

BENCHMARK DOSE AND REFERENCE DOSES OR REFERENCE CONCENTRATIONS

TABLE A-1: EYE MALFORMATIONS

(Narotsky *et al.* 1995)

Model	BMR type	BMR	MLE	BMDL	Log-likelihood	G-O-F P-value	Chi-square	RfD-MLE mg/kg/d	RfD-BMDL mg/kg/d
Exposure			*mg/kg/d*	*mg/kg/d*				100	1
Teramod	**Additional**	**0.1**	**751**	**501**	**-155**			**7.5**	**5.0**
Teramod	Additional	0.05	404	244	-155			4.0	2.4
Teralog	**Additional**	**0.1**	**777**	**505**	**-155**			**7.8**	**5.0**
Teralog	Additional	0.05	486	239	-155			4.9	2.4
AUCTCA			*mg* hr/L*	*mg* hr/L*				30	0.00209
Teramod	**Additional**	**0.1**	**3591**	**2924**	**-155**			**0.25**	**0.20**
Teramod	Additional	0.05	2958	1656	-155			0.21	0.12
Teralog	**Additional**	**0.1**	**3633**	**3023**	**-155**			**0.25**	**0.21**
Teralog	Additional	0.05	3198	1832	-155			0.22	0.13

TABLE A-2: LIVER
(Buben & O'Flaherty 1985)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfD-MLE mg/kg/d	RfD-BMDL mg/kg/d
Exposure					*mg/kg/d*	*mg/kg/d*				100	1.0
Weibull, P0 fixed	One	0.05	0.1	5.9	15	11	23	15	0.0016	0.11	0.08
		0.05	0.05							n/a	n/a
		0.01	0.1	6.2	20	14	355	8	0	0.14	0.10
		0.01	0.05	6.2	9.8	6.9	15	8	0	0.07	0.05
		0.001	0.1	6.6	33	20	967	-2	0	0.23	0.14
		0.001	0.05	6.8	113	64	192	17	0.011	0.81	0.46
K Power, P0 fixed	One per dose group	0.05	0.1	6.7	271	188	387	4	0	1.9	1.3
		0.05	0.05							n/a	n/a
		0.01	0.1	7.1	489	341	700	4	0	3.5	2.4
		0.01	0.05	7.1	343	239	491	4	0	2.5	1.7
		0.001	0.1	7.6	807	562	1155	4	0	5.8	4.0
		0.001	0.05	7.6	647	451	926	4	0	4.6	3.2
AUCTCA					*mg*hr/L*	*mg*hr/L*				10	0.00209
Weibull, P0 fixed	One	0.05	0.1	6.0	540	457	632	21	0.13	0.113	0.096
		0.05	0.05	6.0	465	386	555	21	0.13	0.097	0.081
		0.01	0.1	6.3	640	551	738	21	0.12	0.134	0.12
		0.01	0.05	6.3	562	476	659	21	0.12	0.118	0.10
		0.001	0.1	6.7	773	677	878	20	0.082	0.162	0.14
		0.001	0.05	6.7	695	599	799	20	0.082	0.145	0.13
K Power, P0 fixed	One per dose group	0.05	0.1	6.0	461	372	570	26	0.14	0.096	0.078
		0.05	0.05	6.0	361	283	459	26	0.14	0.076	0.059
		0.01	0.1	6.1	250	196	509	-3	0	0.052	0.041
		0.01	0.05	6.1	175	139	351	-3	0	0.037	0.0291
		0.001	0.1	6.5	412	313	1813	-3	0	0.086	0.066
		0.001	0.05	6.5	330	256	743	-3	0	0.069	0.053

TABLE A-3: LIVER
(Berman *et al.* 1995)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfD-MLE mg/kg/d	RfD-BMDL mg/kg/d
Exposure					*mg/kg/d*	*mg/kg/d*				100	1.0
Weibull, P0 fixed	One	0.05	0.1	6.0	1104	322	91663	-41	0.98	11	3.2
		0.05	0.05	6.0	537	156	44558	-41	0.98	5.4	1.6
		0.01	0.1	7.1	3309	742	n/a	-41	0.99	33	7.4
		0.01	0.05	7.1	1610	361	n/a	-41	0.99	16	3.6
		0.001	0.1	8.4	16136	1960	n/a	-41	0.99	161	20
		0.001	0.05	8.4	8221	1374	n/a	-41	0.99	82	14
K Power, P0 fixed	One per dose group	0.05	0.1	5.1	776	359	8945	-39	0.94	7.8	3.6
		0.05	0.05	5.1	463	215	5342	-39	0.94	4.6	2.1
		0.01	0.1	5.8	1403	650	16170	-39	0.94	14	6.5
		0.01	0.05	5.8	984	456	11344	-39	0.94	9.8	4.6
		0.001	0.1	6.7	2314	1072	26676	-39	0.94	23	11
		0.001	0.05	6.7	1856	859	21392	-39	0.94	19	8.6
AUCTCA					*mg*hr/L*	*mg*hr/L*				10	0.00209
Weibull, P0 fixed	One	0.05	0.1	6.0	419	116	n/a	-41	0.98	0.088	0.0243
		0.05	0.05	6.0	322	57	15331	-41	0.98	0.067	0.0118
		0.01	0.1	7.1	613	308	n/a	-41	0.97	0.128	0.064
		0.01	0.05	7.1	484	152	n/a	-41	0.97	0.101	0.032
		0.001	0.1	8.4	974	510	n/a	-41	0.97	0.204	0.107
		0.001	0.05	8.4	794	473	n/a	-41	0.97	0.166	0.099
K Power, P0 fixed	One per dose group	0.05	0.1	5.0	317	132	2324	-39	0.98	0.066	0.0276
		0.05	0.05	5.0	234	79	1388	-39	0.98	0.049	0.0165
		0.01	0.1	5.7	447	239	4201	-39	0.98	0.093	0.050
		0.01	0.05	5.7	364	167	2947	-39	0.98	0.076	0.035
		0.001	0.1	6.5	599	389	6930	-39	0.98	0.125	0.081
		0.001	0.05	6.5	527	316	5557	-39	0.98	0.110	0.066

TABLE A-4: KIDNEY - ORAL
(Maltoni *et al.* 1986)

Model	BMR type	Compute Threshold	BMR	MLE	BMDL	Log-likelihood	G-O-F P-value	Chi-square	RfD-MLE mg/kg/d	RfD-BMDL mg/kg/d
Exposure				*mg/kg/d*	*mg/kg/d*				100	1
Polynomial Quantal	Extra	Yes	0.1	168	69	-20.7	1	0.00	1.2	0.49
		Yes	0.05	155	52	-20.7	1	0.00	1.1	0.37
		No	0.1	105	70	-21.5	0.38	0.76	0.75	0.50
		No	0.05	73	39	-21.5	0.38	0.76	0.52	0.28
Weibull Quantal	Extra	Yes	0.1	226	84	-20.7	1	0.00	1.6	0.60
		Yes	0.05	217	54	-20.7	1	0.00	1.6	0.39
		No	0.1	226	84	-20.7	1	0.00	1.6	0.60
		No	0.05	217	54	-20.7	1	0.00	1.6	0.39
KTOX				*mg/L*	*mg/L*				30	0.64935
Polynomial Quantal	Extra	Yes	0.1	737	233	-20.7	1	0.00	16	5.0
		Yes	0.05	548	113	-20.7	1	0.00	12	2.5
		No	0.1	673	230	-20.8	0.82	0.05	15	5.0
		No	0.05	470	112	-20.8	0.82	0.05	10	2.4
Weibull Quantal	Extra	Yes	0.1	1396	233	-20.7	1	0.00	30	5.0
		Yes	0.05	1308	113	-20.7	1	0.00	28	2.5
		No	0.1	1396	233	-20.7	1	0.00	30	5.0
		No	0.05	1308	113	-20.7	1	0.00	28	2.5

TABLE A-5HR: HEART RATE
(Arito et al. 1994)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Value	RfC-MLE ppm	RfC-BMDL ppm
Exposure					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	344	54	18	391	-59	0.22	0.42	0.14
		0.05	0.05	344	26	8.5	195	-59	0.22	0.21	0.068
		0.01	0.1	337	124	32	1206	-59	0.23	1.0	0.25
		0.01	0.05	337	61	16	587	-59	0.23	0.48	0.12
		0.001	0.1	328	419	91	7672	-59	0.24	3.3	0.72
		0.001	0.05	328	237	44	3735	-59	0.24	1.9	0.35
K Power, P0 fixed	One per dose group	0.05	0.1	339	155	67	738	-60	0.11	1.2	0.53
		0.05	0.05	339	92	40	441	-60	0.11	0.73	0.32
		0.01	0.1	330	279	122	1334	-60	0.11	2.2	0.97
		0.01	0.05	330	196	85	936	-60	0.11	1.6	0.68
		0.001	0.1	320	461	201	2201	-60	0.11	3.7	1.6
		0.001	0.05	320	370	161	1765	-60	0.11	2.9	1.3

Table A-5hr (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Value	RfC-MLE ppm	RfC-BMDL ppm
AUCTCE					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	1.7730
Weibull, P0 fixed	One	0.05	0.1	342	29	8.8	294	-60	0.13	5.1	1.56
		0.05	0.05	342	14	4.3	143	-60	0.13	2.47	0.76
		0.01	0.1	335	58	15	709	-60	0.17	10.3	2.60
		0.01	0.05	335	28	7.1	345	-60	0.17	5.0	1.27
		0.001	0.1	327	214	37	3705	-59	0.20	38	6.5
		0.001	0.05	327	104	18	1804	-59	0.20	18.5	3.2
K Power, P0 fixed	One per dose group	0.05	0.1	337	86	35	577	-60	0.070	15.2	6.2
		0.05	0.05	337	51	21	345	-60	0.070	9.1	3.7
		0.01	0.1	327	155	64	1043	-60	0.070	27.5	11.3
		0.01	0.05	327	109	45	732	-60	0.070	19.3	7.9
		0.001	0.1	317	256	105	1721	-60	0.070	45	18.6
		0.001	0.05	317	205	84	1380	-60	0.070	36	14.9
AUCTCH					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.319
Weibull, P0 fixed	One	0.05	0.1	345	9.0	2.3	41	-59	0.31	0.29	0.074
		0.05	0.05	345	5.0	1.1	25	-59	0.31	0.160	0.036
		0.01	0.1	337	20	4.7	130	-59	0.32	0.63	0.151
		0.01	0.05	337	12	2.3	63	-59	0.32	0.39	0.073
		0.001	0.1	328	44	16	772	-59	0.33	1.41	0.50
		0.001	0.05	328	30	7.8	376	-59	0.33	0.97	0.248
K Power, P0 fixed	One per dose group	0.05	0.1	342	16	7.8	58	-59	0.23	0.51	0.249
		0.05	0.05	342	9.6	4.7	35	-59	0.23	0.31	0.149
		0.01	0.1	333	29	14	104	-59	0.23	0.93	0.45
		0.01	0.05	333	20	9.9	73	-59	0.23	0.65	0.32
		0.001	0.1	324	48	23	172	-59	0.23	1.53	0.74
		0.001	0.05	324	38	19	138	-59	0.23	1.23	0.60

Table A-5hr (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Value	RfC-MLE ppm	RfC-BMDL ppm
CV					*mg/L*	*mg/L*	*mg/L*			10	52
Weibull, P0 fixed	One	0.05	0.1	341	4.0	1.2	49	-60	0.11	20.8	6.1
		0.05	0.05	341	1.9	0.57	24	-60	0.11	10.1	3.0
		0.01	0.1	335	7.7	1.9	108	-60	0.14	40	9.9
		0.01	0.05	335	3.8	0.93	53	-60	0.14	19.6	4.8
		0.001	0.1	327	27	4.6	495	-60	0.18	138	23.7
		0.001	0.05	327	13	2.2	241	-60	0.18	67	11.5
K Power, P0 fixed	One per dose group	0.05	0.1	336	11	4.7	88	-60	0.062	60	24.2
		0.05	0.05	336	6.8	2.8	53	-60	0.062	36	14.5
		0.01	0.1	327	21	8.4	159	-60	0.062	108	44
		0.01	0.05	327	15	5.9	112	-60	0.062	76	31
		0.001	0.1	316	34	14	262	-60	0.062	178	72
		0.001	0.05	316	27	11	210	-60	0.062	142	58
CTCOH					*mg/L*	*mg/L*	*mg/L*			10	7.63
Weibull, P0 fixed	One	0.05	0.1	345	1.3	0.26	3.7	-59	0.40	0.96	0.202
		0.05	0.05	345	0.81	0.13	2.7	-59	0.40	0.62	0.098
		0.01	0.1	338	2.3	0.58	11	-59	0.41	1.74	0.44
		0.01	0.05	338	1.6	0.28	5.4	-59	0.41	1.21	0.214
		0.001	0.1	329	4.2	1.9	62	-59	0.42	3.2	1.44
		0.001	0.05	329	3.1	1.1	30	-59	0.42	2.40	0.81
K Power, P0 fixed	One per dose group	0.05	0.1	343	1.5	0.78	4.8	-59	0.35	1.15	0.59
		0.05	0.05	343	0.90	0.46	3.3	-59	0.35	0.68	0.35
		0.01	0.1	335	2.7	1.4	8.6	-59	0.35	2.07	1.07
		0.01	0.05	335	1.9	0.99	6.1	-59	0.35	1.45	0.75
		0.001	0.1	325	4.5	2.3	14	-59	0.35	3.4	1.77
		0.001	0.05	325	3.6	1.9	11	-59	0.35	2.74	1.42

TABLE A-5W: WAKEFULNESS
(Arito et al. 1994)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfC-MLE ppm	RfC-BMDL ppm
Exposure					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	86	12	5.4	54	-58	0.26	0.10	0.043
		0.05	0.05	86	5.9	2.6	33	-58	0.26	0.047	0.021
		0.01	0.1	80	23	7.8	106	-58	0.28	0.18	0.062
		0.01	0.05	80	12	3.8	68	-58	0.28	0.094	0.030
		0.001	0.1	71	73	15	277	-58	0.28	0.58	0.12
		0.001	0.05	71	43	7.5	167	-58	0.28	0.35	0.059
K Power, P0 fixed	One per dose group	0.05	0.1	64	140	72	329	-57	0.022	1.1	0.57
		0.05	0.05	64	83	43	199	-57	0.022	0.66	0.34
		0.01	0.1	51	253	130	594	-57	0.022	2.0	1.0
		0.01	0.05	51	177	91	417	-57	0.022	1.4	0.72
		0.001	0.1	36	417	214	981	-57	0.022	3.3	1.7
		0.001	0.05	36	334	171	786	-57	0.022	2.7	1.4

Table A-5W (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfC-MLE ppm	RfC-BMDL ppm
AUCTCE					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	1.773
Weibull, P0 fixed	One	0.05	0.1	83	6.4	2.8	23	-59	0.10	1.14	0.50
		0.05	0.05	83	3.1	1.4	12	-59	0.10	0.55	0.24
		0.01	0.1	78	9.8	3.8	41	-58	0.17	1.73	0.67
		0.01	0.05	78	4.8	1.8	22	-58	0.17	0.84	0.32
		0.001	0.1	71	21	6.2	132	-58	0.22	3.7	1.1
		0.001	0.05	71	10	3.0	66	-58	0.22	1.81	0.53
K Power, P0 fixed	One per dose group	0.05	0.1	60	80	41	198	-58	0.0075	14.1	7.2
		0.05	0.05	60	48	24	118	-58	0.0075	8.4	4.3
		0.01	0.1	46	144	74	358	-58	0.0075	25.6	13
		0.01	0.05	46	101	52	251	-58	0.0075	17.9	9.1
		0.001	0.1	30	238	121	590	-58	0.0075	42	22
		0.001	0.05	30	191	97	473	-58	0.0075	34	17
AUCTCH					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.319
Weibull, P0 fixed	One	0.05	0.1	87	2.9	0.74	11	-58	0.48	0.093	0.024
		0.05	0.05	87	1.7	0.36	8.0	-58	0.48	0.053	0.011
		0.01	0.1	80	6.3	1.2	18	-58	0.47	0.202	0.040
		0.01	0.05	80	4.0	0.61	13	-58	0.47	0.127	0.019
		0.001	0.1	72	14	3.5	34	-58	0.47	0.44	0.11
		0.001	0.05	72	9.7	1.7	24	-58	0.47	0.31	0.054
K Power, P0 fixed	One per dose group	0.05	0.1	72	13	6.9	28	-55	0.15	0.41	0.22
		0.05	0.05	72	7.6	4.1	17	-55	0.15	0.244	0.13
		0.01	0.1	60	23	12	51	-55	0.15	0.74	0.40
		0.01	0.05	60	16	8.7	36	-55	0.15	0.52	0.28
		0.001	0.1	46	38	20	84	-55	0.15	1.22	0.65
		0.001	0.05	46	31	16	68	-55	0.15	0.98	0.52

Table A-5W (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfC-MLE ppm	RfC-BMDL ppm
CV					*mg/L*	*mg/L*	*mg/L*			10	52
Weibull, P0 fixed	One	0.05	0.1	82	0.89	0.38	3.2	-59	0.062	4.6	2.0
		0.05	0.05	82	0.43	0.19	1.7	-59	0.062	2.24	0.97
		0.01	0.1	76	1.3	0.50	5.4	-59	0.13	6.8	2.6
		0.01	0.05	76	0.63	0.24	2.8	-59	0.13	3.3	1.3
		0.001	0.1	70	2.7	0.80	17	-58	0.20	13.9	4.1
		0.001	0.05	70	1.3	0.39	8.1	-58	0.20	6.8	2.0
K Power, P0 fixed	One per dose group	0.05	0.1	59	11	5.4	27	-59	0.0058	55	28
		0.05	0.05	59	6.4	3.2	16	-59	0.0058	33	17
		0.01	0.1	45	19	9.8	49	-59	0.0058	100	51
		0.01	0.05	45	14	6.9	34	-59	0.0058	70	36
		0.001	0.1	29	32	16	80	-59	0.0058	165	84
		0.001	0.05	29	25	13	64	-59	0.0058	133	68
CTCOH					*mg/L*	*mg/L*	*mg/L*			10	7.63
Weibull, P0 fixed	One	0.05	0.1	88	0.51	0.091	1.5	-57	0.68	0.39	0.069
		0.05	0.05	88	0.32	0.044	1.2	-57	0.68	0.247	0.034
		0.01	0.1	80	0.93	0.18	2.1	-57	0.67	0.71	0.14
		0.01	0.05	80	0.65	0.09	1.7	-57	0.67	0.49	0.066
		0.001	0.1	72	1.7	0.58	3.4	-57	0.67	1.30	0.44
		0.001	0.05	72	1.3	0.33	2.6	-57	0.67	0.98	0.25
K Power, P0 fixed	One per dose group	0.05	0.1	77	1.1	0.61	2.2	-53	0.39	0.80	0.47
		0.05	0.05	77	0.63	0.37	1.3	-53	0.39	0.48	0.28
		0.01	0.1	65	1.9	1.1	3.9	-53	0.39	1.45	0.85
		0.01	0.05	65	1.3	0.78	2.8	-53	0.39	1.02	0.59
		0.001	0.1	52	3.1	1.8	6.5	-53	0.39	2.40	1.4
		0.001	0.05	52	2.5	1.5	5.2	-53	0.39	1.92	1.1

TABLE A-5SWS: SLOW-WAVE SLEEP
(Arito et al. 1994)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
Exposure					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	322	12	5.3	61	-59	0.080	0.094	0.042
		0.05	0.05	322	5.8	2.6	39	-59	0.080	0.046	0.021
		0.01	0.1	330	32	8.1	117	-59	0.078	0.26	0.064
		0.01	0.05	330	18	3.9	79	-59	0.078	0.14	0.031
		0.001	0.1	339	88	19	270	-59	0.078	0.69	0.15
		0.001	0.05	339	56	9.4	176	-59	0.078	0.44	0.075
K Power, PO fixed	One per dose group	0.05	0.1	325	89	43	218	-61	0.011	0.71	0.34
		0.05	0.05	335	53	26	143	-61	0.011	0.42	0.20
		0.01	0.1	345	161	78	394	-61	0.011	1.3	0.62
		0.01	0.05	345	113	55	277	-61	0.011	0.89	0.43
		0.001	0.1	357	265	129	650	-61	0.011	2.1	1.0
		0.001	0.05	357	212	103	522	-61	0.011	1.7	0.82

Table A-5SWS (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
AUCTCE					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	1.773
Weibull, P0 fixed	One	0.05	0.1	325	6.1	2.7	22	-60	0.035	1.09	0.48
		0.05	0.05	325	3.0	1.3	13	-60	0.035	0.53	0.23
		0.01	0.1	331	9.7	3.7	42	-59	0.051	1.71	0.66
		0.01	0.05	331	4.7	1.8	24	-59	0.051	0.83	0.32
		0.001	0.1	339	27	6.5	137	-59	0.055	4.8	1.1
		0.001	0.05	339	14	3.1	73	-59	0.055	2.55	0.56
K Power, PO fixed	One per dose group	0.05	0.1	341	53	25	136	-62	0.0032	9.4	4.4
		0.05	0.05	341	32	15	127	-62	0.0032	5.6	2.6
		0.01	0.1	352	96	45	245	-62	0.0032	17.0	8.0
		0.01	0.05	352	67	31	172	-62	0.0032	11.9	5.6
		0.001	0.1	365	158	74	404	-62	0.0032	28.0	13
		0.001	0.05	365	127	59	324	-62	0.0032	22.4	11
AUCTCH					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.319
Weibull, P0 fixed	One	0.05	0.1	321	4.1	0.82	12	-58	0.17	0.130	0.026
		0.05	0.05	321	2.5	0.40	9.1	-58	0.17	0.081	0.013
		0.01	0.1	329	7.9	1.7	19	-58	0.17	0.253	0.055
		0.01	0.05	329	5.4	0.84	15	-58	0.17	0.171	0.027
		0.001	0.1	337	15	5.6	32	-58	0.17	0.49	0.18
		0.001	0.05	337	11	3.3	25	-58	0.17	0.37	0.10
K Power, P0 fixed	One per dose group	0.05	0.1	327	8.2	4.6	18	-58	0.077	0.26	0.15
		0.05	0.05	327	4.9	2.7	12	-58	0.077	0.155	0.087
		0.01	0.1	336	15	8.3	33	-58	0.077	0.47	0.26
		0.01	0.05	336	10	5.8	23	-58	0.077	0.33	0.19
		0.001	0.1	347	24	14	54	-58	0.077	0.78	0.44
		0.001	0.05	347	20	11	43	-58	0.077	0.62	0.35

Table A-5SWS (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
CV					*mg/L*	*mg/L*	*mg/L*			10	52
Weibull, P0 fixed	One	0.05	0.1	326	0.84	0.37	3.0	-60	0.024	4.4	1.9
		0.05	0.05	326	0.41	0.18	1.6	-60	0.024	2.11	0.93
		0.01	0.1	332	1.3	0.49	5.3	-60	0.040	6.6	2.5
		0.01	0.05	332	0.62	0.24	2.9	-60	0.040	3.2	1.2
		0.001	0.1	340	2.9	0.81	18	-59	0.051	15.2	4.2
		0.001	0.05	340	1.5	0.39	8.8	-59	0.051	7.6	2.0
K Power, P0 fixed	One per dose group	0.05	0.1	342	7.2	3.4	19	-62	0.0024	37	17
		0.05	0.05	342	4.3	2.0	15	-62	0.0024	22.2	10
		0.01	0.1	354	13	6.1	34	-62	0.0024	67	32
		0.01	0.05	354	9.1	4.3	24	-62	0.0024	47	22
		0.001	0.1	367	21	10	55	-62	0.0024	111	52
		0.001	0.05	367	17	8.0	44	-62	0.0024	89	42
CTCOH					*mg/L*	*mg/L*	*mg/L*			10	7.63
Weibull, P0 fixed	One	0.05	0.1	320	0.67	0.14	1.6	-58	0.30	0.51	0.11
		0.05	0.05	320	0.47	0.070	1.3	-58	0.30	0.36	0.053
		0.01	0.1	328	1.1	0.35	2.2	-58	0.30	0.85	0.27
		0.01	0.05	328	0.83	0.20	1.8	-58	0.30	0.63	0.15
		0.001	0.1	337	1.8	0.85	3.2	-58	0.30	1.41	0.65
		0.001	0.05	337	1.5	0.57	2.6	-58	0.30	1.12	0.43
K Power, P0 fixed	One per dose group	0.05	0.1	323	0.71	0.44	1.4	-56	0.13	0.54	0.34
		0.05	0.05	323	0.42	0.26	0.9	-56	0.13	0.32	0.20
		0.01	0.1	332	1.3	0.79	2.5	-56	0.13	0.98	0.61
		0.01	0.05	332	0.90	0.56	1.8	-56	0.13	0.69	0.43
		0.001	0.1	342.7	2.1	1.3	4.2	-56	0.13	1.6	1.00
		0.001	0.05	342.7	1.7	1.1	3.4	-56	0.13	1.3	0.80

TABLE A-5PS: PARADOXICAL SLEEP
(Arito et al. 1994)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
Exposure					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	66	1978	92	n/a	-50	0.16	16	0.73
		0.05	0.05	66	961	45	n/a	-50	0.16	7.6	0.36
		0.01	0.1	61	6478	223	n/a	-50	0.16	51	1.8
		0.01	0.05	61	3153	109	n/a	-50	0.16	25	0.86
		0.001	0.1	55	15000	575	n/a	-50	0.16	119	4.6
		0.001	0.05	55	15000	420	n/a	-50	0.16	119	3.3
K Power, P0 fixed	One per dose group	0.05	0.1	75	206	63	n/a	-44	0.16	1.6	0.50
		0.05	0.05	75	123	38	n/a	-44	0.16	1.0	0.30
		0.01	0.1	74	373	115	n/a	-44	0.16	3.0	0.91
		0.01	0.05	74	262	81	n/a	-44	0.16	2.1	0.64
		0.001	0.1	72	615	189	n/a	-44	0.16	4.9	1.5
		0.001	0.05	72	493	152	n/a	-44	0.16	3.9	1.2

Table 5PS (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
AUCTCE					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	1.773
Weibull, P0 fixed	One	0.05	0.1	65	4684	54	n/a	-50	0.16	831	9.5
		0.05	0.05	65	2277	26	n/a	-50	0.16	404	4.6
		0.01	0.1	60	7028	126	n/a	-50	0.15	1246	22
		0.01	0.05	60	7028	62	n/a	-50	0.16	1246	11
		0.001	0.1	54	7028	319	n/a	-50	0.16	1246	57
		0.001	0.05	54	7028	242	n/a	-50	0.16	1246	43
K Power, PO fixed	One per dose group	0.05	0.1	75	112	28	n/a	-44	0.15	19.9	5.0
		0.05	0.05	75	67	17	n/a	-44	0.15	11.9	3.0
		0.01	0.1	74	203	51	n/a	-44	0.15	36	9.0
		0.01	0.05	74	142	35	n/a	-44	0.15	25.2	6.3
		0.001	0.1	72	334	83	n/a	-44	0.15	59	15
		0.001	0.05	72	268	67	n/a	-44	0.15	48	12
AUCTCH					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.319
Weibull, P0 fixed	One	0.05	0.1	66	103	10	n/a	-50	0.17	3.3	0.33
		0.05	0.05	66	50	5.03	n/a	-50	0.17	1.60	0.16
		0.01	0.1	61	345	26	n/a	-50	0.17	11.0	0.84
		0.01	0.05	61	168	13	n/a	-50	0.17	5.3	0.41
		0.001	0.1	56	1875	58	n/a	-50	0.17	60	1.9
		0.001	0.05	56	1129	44	n/a	-50	0.17	36	1.4
K Power, P0 fixed	One per dose group	0.05	0.1	75	24	8.7	n/a	-44	0.17	0.76	0.28
		0.05	0.05	75	14	5.2	n/a	-44	0.17	0.46	0.17
		0.01	0.1	74	43	16	n/a	-44	0.17	1.38	0.50
		0.01	0.05	74	30	11	n/a	-44	0.17	0.97	0.35
		0.001	0.1	73	71	26	n/a	-44	0.17	2.28	0.83
		0.001	0.05	73	57	21	n/a	-44	0.17	1.83	0.66

Table 5PS (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-Values	RfC-MLE ppm	RfC-BMDL ppm
CV					*mg/L*	*mg/L*	*mg/L*			10	52
Weibull, P0 fixed	One	0.05	0.1	65	895	7.4	n/a	-50	0.15	4657	38
		0.05	0.05	65	895	3.6	n/a	-50	0.15	4657	19
		0.01	0.1	60	895	18	n/a	-50	0.16	4657	92
		0.01	0.05	60	895	8.6	n/a	-50	0.16	4657	45
		0.001	0.1	54	895	45	n/a	-50	0.16	4657	232
		0.001	0.05	54	895	34	n/a	-50	0.16	4657	174
K Power, P0 fixed	One per dose group	0.05	0.1	75	15	3.5	n/a	-44	0.15	80	18
		0.05	0.05	75	9.1	2.1	n/a	-44	0.15	47	11
		0.01	0.1	74	28	6.3	n/a	-44	0.15	144	33
		0.01	0.05	74	19	4.5	n/a	-44	0.15	101	23
		0.001	0.1	72	46	10	n/a	-44	0.15	237	54
		0.001	0.05	72	37	8.4	n/a	-44	0.15	190	44
CTCOH					*mg/L*	*mg/L*	*mg/L*			10	7.63
Weibull, P0 fixed	One	0.05	0.1	67	7.3	1.0	n/a	-50	0.18	5.5	0.79
		0.05	0.05	67	3.9	0.50	n/a	-50	0.18	2.97	0.38
		0.01	0.1	62	18	2.7	n/a	-50	0.18	13.7	2.1
		0.01	0.05	62	10	1.3	n/a	-50	0.18	7.7	1.0
		0.001	0.1	56	58	5.1	n/a	-50	0.19	44	3.9
		0.001	0.05	56	35	4.1	n/a	-50	0.19	26.3	3.1
K Power, P0 fixed	One per dose group	0.05	0.1	75	2.4	0.92	n/a	-44	0.18	1.82	0.70
		0.05	0.05	75	1.4	0.55	n/a	-44	0.18	1.08	0.42
		0.01	0.1	74	4.3	1.7	n/a	-44	0.18	3.3	1.3
		0.01	0.05	74	3.0	1.2	n/a	-44	0.18	2.30	0.89
		0.001	0.1	73	7.1	2.8	n/a	-44	0.18	5.4	2.1
		0.001	0.05	73	5.7	2.2	n/a	-44	0.18	4.3	1.7

TABLE A-6: LIVER
(Kjellstrand et al. 1983a)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfC-MLE ppm	RfC-BMDL ppm
Exposure, female mice					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	139	30	21	42	-262	0.87	0.99	0.69
		0.05	0.05	139	22	15	32	-262	0.87	0.72	0.49
		0.01	0.1	150	44	32	59	-262	0.80	1.5	1.1
		0.01	0.05	150	34	24	47	-262	0.80	1.1	0.78
		0.001	0.1	163	67	51	87	-263	0.65	2.2	1.7
		0.001	0.05	163	54	40	72	-263	0.65	1.8	1.3
K Power, P0 fixed	One per dose group	0.05	0.1	129	15	12	23	-243	0.83	0.52	0.40
		0.05	0.05	129	9.4	7.1	15	-243	0.83	0.31	0.24
		0.01	0.1	135	27	21	38	-243	0.83	0.91	0.72
		0.01	0.05	135	19	15	28	-243	0.83	0.65	0.50
		0.001	0.1	143	45	35	59	-243	0.83	1.5	1.2
		0.001	0.05	143	36	28	48	-243	0.83	1.2	0.95
AUCTCA, female mice					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.0043
Weibull, P0 fixed	One	0.05	0.1	139	2405	2075	2769	-263	0.52	1.0	0.9
		0.05	0.05	139	2103	1782	2460	-263	0.52	0.9	0.8
		0.01	0.1	150	2851	2496	3234	-263	0.40	1.2	1.1
		0.01	0.05	150	2541	2191	2924	-263	0.40	1.1	0.9
		0.001	0.1	163	3443	3062	3839	-264	0.27	1.5	1.3
		0.001	0.05	163	3136	2753	3538	-264	0.27	1.4	1.2
K Power, P0 fixed	One per dose group	0.05	0.1	128	1880	1585	2197	-244	0.28	0.8	0.7
		0.05	0.05	128	1530	1256	1827	-244	0.28	0.7	0.5
		0.01	0.1	135	2382	2067	2717	-244	0.28	1.0	0.9
		0.01	0.05	135	2067	1763	2392	-244	0.28	0.9	0.8
		0.001	0.1	143	2910	2583	3259	-244	0.28	1.3	1.1
		0.001	0.05	143	2664	2342	3007	-244	0.28	1.2	1.0

Table A-6 (cont.)

Model	Standard Deviation	P_0	BMR	X_0	MLE	BMDL	Upper Bound	Log-likelihood	G-O-F P-value	RfC-MLE ppm	RfC-BMDL ppm
Exposure, male mice					*ppm*	*ppm*	*ppm*			30	1.0
Weibull, P0 fixed	One	0.05	0.1	159	5.2	2.3	10	-270	0.020	0.17	0.077
		0.05	0.05	159	2.8	1.1	6.2	-270	0.020	0.095	0.038
		0.01	0.1	171	11	5.4	20	-270	0.013	0.36	0.18
		0.01	0.05	171	6.5	2.9	13	-270	0.013	0.22	0.10
		0.001	0.1	185	25	14	42	-271	0.0081	0.82	0.45
		0.001	0.05	185	16	8.1	29	-271	0.0081	0.54	0.27
K Power, P0 fixed	One per dose group	0.05	0.1	154	17	13	22	-271	0	0.55	0.44
		0.05	0.05	154	9.9	8.0	13	-271	0	0.33	0.27
		0.01	0.1	162	30	24	40	-271	0	1.0	0.80
		0.01	0.05	162	21	17	28	-271	0	0.70	0.56
		0.001	0.1	172	50	40	66	-271	0	1.7	1.3
		0.001	0.05	172	40	32	53	-271	0	1.3	1.1
AUCTCA, male mice					*mg*hr/L*	*mg*hr/L*	*mg*hr/L*			10	0.0043
Weibull, P0 fixed	One	0.05	0.1	159	1198	852	1595	-272	0.0039	0.52	0.37
		0.05	0.05	159	929	628	1285	-272	0.0039	0.40	0.27
		0.01	0.1	172	1635	1214	2111	-272	0.0025	0.71	0.53
		0.01	0.05	172	1315	935	1755	-272	0.0025	0.57	0.40
		0.001	0.1	187	2305	1789	2874	-273	0.0014	1.0	0.77
		0.001	0.05	187	1930	1445	2475	-273	0.0014	0.84	0.63
K Power, P0 fixed	One per dose group	0.05	0.1	151	1043	697	1361	-258	0.0003	0.45	0.30
		0.05	0.05	151	743	459	1012	-258	0.0003	0.32	0.20
		0.01	0.1	159	1538	1122	1917	-258	0.0003	0.67	0.49
		0.01	0.05	159	1219	844	1560	-258	0.0003	0.53	0.37
		0.001	0.1	169	2136	1673	2572	-258	0.0003	0.92	0.72
		0.001	0.05	169	1848	1404	2258	-258	0.0003	0.80	0.61

TABLE A-7: KIDNEY-INHALATION
(Maltoni et al. 1986)

Model	BMR type	Compute Threshold	BMR	MLE	BMDL	Log-likelihood	G-O-F P-value	Chi-square	RfC-MLE ppm	RfC-BMDL ppm
Exposure				*ppm*	*ppm*				30	1
Polynomial Quantal	Extra	Yes	0.1	238	176	-93	1.0	0.0	1.7	1.2
		Yes	0.05	197	138	-93	1.0	0.0	1.4	0.96
		No	0.1	245	203	-94	0.59	1.1	1.7	1.4
		No	0.05	193	148	-94	0.59	1.1	1.3	1.0
Weibull Quantal	Extra	Yes	0.1	241	201	-93	1.0	0.00	1.7	1.4
		Yes	0.05	201	162	-93	1.0	0.00	1.4	1.1
		No	0.1	241	202	-94	0.33	0.95	1.7	1.4
		No	0.05	189	150	-94	0.33	0.95	1.3	1.0
KTOX				*mg/L*	*mg/L*				30	1.351
Polynomial Quantal	Extra	Yes	0.1	585	353	-93	1.0	0.00	26	16
		Yes	0.05	356	192	-93	1.0	0.00	16	9
		No	0.1	623	370	-93	0.54	0.37	28	17
		No	0.05	384	182	-93	0.54	0.37	17	8
Weibull Quantal	Extra	Yes	0.1	625	430	-93	1.0	0.00	28	19
		Yes	0.05	424	261	-93	1.0	0.00	19	12
		No	0.1	623	436	-93	0.77	0.09	28	20
		No	0.05	409	258	-93	0.77	0.09	18	12

APPENDIX B

TABLES OF TABLES

DOSE METRICS FOR BENCHMARK DOSE CALCULATIONS

TABLE B-1: EYE MALFORMATIONS
(Narotsky et al. 1995)

Values at Time T						*Peak Values for*					
BW	Dose	Time		AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
kg	*mg/kg/day*	*hours*	*days*								
0.175	10	24	1	0.11	10	34	1.70	0.051	2.3	0.8	0.05
0.175	32			0.62	31	71	5.70	0.5	4.6	2.7	0.43
0.175	101			13	73	133	14	8.6	8.4	3.7	8
0.175	320			88	129	211	26	39	13	3.8	36
0.175	475			150	151	240	30	60	14	3.8	55
0.175	633			215	168	260	34	82	15	3.8	75
0.175	844			304	186	280	37	111	16	3.8	102
0.175	1125			426	204	300	41	149	18	3.8	138
0.175	10	216	9	0.95	88	366	15	0.051	2.8	0.83	0.05
0.175	32			5.61	276	769	51	0.46	5.7	2.7	0.43
0.175	101			117	656	1475	128	8.6	10	3.7	7.9
0.175	320			796	1161	2420	231	39	16	3.8	36
0.175	475			1348	1362	2784	272	60	18	3.8	55
0.175	633			1936	1515	3059	303	82	19	3.8	75
0.175	844			2741	1674	3341	336	111	21	3.8	102
0.175	1125			3835	1838	3628	369	150	22	3.81	138
0.175	10	240	10	1.06	98	408	17	0.051	2.8	0.83	0.05
0.175	32			6.23	307	857	57	0.46	5.7	2.7	0.43
0.175	101			130	729	1644	143	8.6	10	3.7	7.9
0.175	320			884	1290	2697	257	39	16	3.8	36
0.175	475			1498	1513	3104	302	60	18	3.8	55
0.175	633			2151	1683	3412	337	82	19	3.8	75
0.175	844			3045	1860	3726	373	111	21	3.8	102
0.175	1125			4261	2042	4046	410	150	22	3.8	138
average daily value for BMD calculations											
	Dose mg/kg/d			AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
	10			0.11	10	41	1.7				
	32			0.62	31	86	5.7	0.46	5.7	2.7	0.43
	101			13	73	164	14	8.6	10	3.7	7.9
	320			88	129	270	26	39	16	3.8	36
	475			150	151	310	30	60	18	3.8	55
	633			215	168	341	34	82	19	3.8	75
	844			305	186	373	37	111	21	3.8	102
	1125			426	204	405	41	150	22	3.8	138

TABLE B-2: LIVER
(Buben & O'Flaherty 1985)

				Values at Time T:				*Peak Values for:*			
BW	Dose	Time		AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
kg	*mg/kg/day*	*hours*	*days*								
0.04	100	168	7	2.2	488	3553	69				
0.04		336	14	20	1094	7564	161				
0.04		504	21	38	1700	11574	252				
0.04		672	28	56	2306	15584	344				
0.04		840	35	74	2911	19595	436				
0.04		1008	42	92	3517	23605	528	18	67	17	16
0.04	200	168	7	31	839	4882	138				
0.04		336	14	114	1804	10265	301				
0.04		504	21	197	2770	15647	464				
0.04		672	28	280	3735	21030	627				
0.04		840	35	363	4701	26412	790				
0.04		1008	42	446	5666	31795	953	49	82	17	44
0.04	400	168	7	151	1212	6333	212				
0.04		336	14	428	2557	13196	451				
0.04		504	21	706	3903	20059	689				
0.04		672	28	984	5249	26922	928				
0.04		840	35	1262	6594	33786	1167				
0.04		1008	42	1540	7940	40649	1405	111	97	17	99
0.04	800	168	7	457	1605	7871	290				
0.04		336	14	1194	3349	16292	608				
0.04		504	21	1931	5093	24713	926				
0.04		672	28	2667	6838	33135	1244				
0.04		840	35	3404	8582	41556	1562				
0.04		1008	42	4140	10326	49977	1880	235	112	17	210
0.04	1600	168	7	1143	2014	9467	372				
0.04		336	14	2871	4171	19499	772				
0.04		504	21	4598	6328	29530	1172				
0.04		672	28	6326	8484	39562	1572				
0.04		840	35	8053	10641	49593	1972				
0.04		1008	42	9781	12798	59624	2372	482	127	17	431

Table B-2 (cont.)

		Values at Time T:						*Peak Values for:*			
BW	Dose	Time		AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
kg	*mg/kg/day*	*hours*	*days*								
0.04	2400	168	7	1861	2259	10420	420				
0.04		336	14	4611	4662	21410	870				
0.04		504	21	7361	7065	32400	1319				
0.04		672	28	10111	9468	43390	1768				
0.04		840	35	12861	11871	54381	2217				
0.04		1008	42	15611	14274	65371	2667	730	137	17	653
0.04	3200	168	7	2591	2435	11102	455				
0.04		336	14	6377	5014	22778	940				
0.04		504	21	10164	7594	34454	1424				
0.04		672	28	13950	10174	46131	1909				
0.04		840	35	17736	12753	57807	2393				
0.04		1008	42	21522	15333	69483	2878	978	145	17	874

Average Daily AUC For BMD Calculation											
	Dose mg/kg/d			AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
	100			2.6	87	573	13	18	67	17	16
	200			12	138	769	23	49	82	17	44
	400			40	192	980	34	111	97	17	99
	800			105	249	1203	45	235	112	17	210
	1600			247	308	1433	57	482	127	17	431
	2400			393	343	1570	64	730	137	17	653
	3200			541	369	1668	69	978	145	17	874

TABLE B-3: LIVER
(Berman et al. 1995)

		Values at Time T:					*Peak Values for:*				
BW	Dose	Time		AUCB	AMET	AUCTCA	AUCTCH	CV	CTCA	CTCOH	CA
kg	*mg/kg/day*	*hours*	*days*	*mg*hr/L*	*mg/L*	*mg*hr/L*	*mg*hr/L*	*mg/L*	*mg/L*	*mg/L*	*mg/L*
0.23	1500	24	1	631	219	319	47	212	18	3.81	196
0.23	50	312	13	34	581	1527	119	2.30	7.45	3.48	2.12
0.23	150			395	1148	2683	241	16	12	3.76	15
0.23	500			2238	1960	4303	417	67	19	3.80	62
0.23	1500			8206	2848	5977	610	213	24	3.81	196
0.23	50	336	14	37	625	1647	128	2.30	7.45	3.48	2.12
0.23	150			426	1236	2894	260	16	12	3.76	15
0.23	500			2410	2111	4643	449	67	19	3.80	62
0.23	1500			8838	3068	6452	657	213	24	3.81	196

Average Daily Value For BMD Calculations											
	Dose mg/kg/d			AUCB mg&hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
	50			2.6	45	118	9.1	2.30	7.45	3.48	2.12
	150			30	88	207	19	16	12	3.76	15
	500			172	151	332	32	67	19	3.80	62
	1500			631	219	461	47	213	24	3.81	196

TABLE B-4: NEUROLOGICAL - INHALATION
(Arito et al. 1994)

Values at Time T:								*Peak Values for:*			
BW	Dose	Time		AUCB	AMET	AUCTCA	AUCTCH	CV	CTCA	CTCOH	CA
kg	*ppm*	*hours*	*days*	*mg*hr/L*	*mg/L*	*mg*hr/L*	*mg*hr/L*	*mg/L*	*mg/L*	*mg/L*	*mg/L*
0.17	50	168	7	67	265	777	48				
0.17		336	14	135	531	1555	96				
0.17		504	21	202	796	2333	144				
0.17		672	28	270	1061	3111	192				
0.17		840	35	337	1327	3888	240				
0.17		1008	42	404	1592	4666	288	1.38	10	1.20	1.67
0.17	100	168	7	140	519	1196	99				
0.17		336	14	281	1037	2394	198				
0.17		504	21	421	1556	3592	296				
0.17		672	28	561	2074	4789	395				
0.17		840	35	702	2593	5987	494				
0.17		1008	42	842	3111	7185	593	2.95	14	2.46	3.51
0.17	300	168	7	703	943	1942	187				
0.17		336	14	1406	1885	3886	375				
0.17		504	21	2108	2828	5831	562				
0.17		672	28	2811	3770	7775	750				
0.17		840	35	3514	4713	9720	937				
0.17		1008	42	4217	5655	11664	1125	18	21	3.74	18.83

Steady State Average Daily Values (AUC) And Maximum Concentrations											
	Dose ppm			AUCB mg*hr/L	AMET mg/L	AUCTCA mg*hr/L	AUCTCH mg*hr/L	CV mg/L	CTCA mg/L	CTCOH mg/L	CA mg/L
	50			13	53	156	9.6	1.38	10	1.20	1.67
	100			28	104	240	20	2.95	14	2.46	3.51
	300			141	189	389	37	18	21	3.74	18.83

TABLE B-5: LIVER - INHALATION
(Kjellstrand et al. 1983a)

Daily Average for:				Peak Values for:					
BW	Dose	AUCB	AMET	AUCTCA	AUCTCH	CV	CTCA	CTCOH	CA
kg	*ppm*	*mg*hr/L*	*mg/L*	*mg*hr/L*	*mg*hr/L*	*mg/L*	*mg/L*	*mg/L*	*mg/L*
0.03	37	29	196	2789	18	1.0	116	0.75	1.2
0.03	75	58	397	3620	42	2.0	151	1.7	2.4
0.03	150	117	792	4688	96	4.1	195	4.0	4.9
0.03	300	238	1561	6466	236	8.4	269	10	10

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APPENDIX C
ABBREVIATIONS

Abbreviations

AMET	amount metabolized by oxidative pathway per unit body weight
AUC	area under the concentration curve
AUCTCA	AUC for TCA in blood
AUCTCE	AUC for TCE in blood
AUCTCH	AUC for TCOH in blood
BM	body weight
BMD	benchmark dose
BMDL	lower bound on BMD
BMR	risk associated with BMD
CATCE	peak arterial concentration of TCE
CTCA	peak blood concentration of TCA
CTCOH	peak blood concentration of TCOH
CVTCE	peak venous concentration of TCE
DCA	dichloroachetate
DCVC	dichlorovinylcysteine
HEC	human equivalent concentration
KTOX	dose metric for kidney toxicity – amount per diney volume of reactive metabolite formed by the DCVC pathway
LOAEL	lowest-observed-adverse-effect-level
LW	liver weight
LW/BW	liver weight to body weight ratio
MLE	maximum likelihood estimate
NOAEL	no-observed adverse-effect-level
PBPK	physiologically based pharmacokinetic
RfC	reference concentration
RfD	reference dose
TCA	trichoroachetate
TCE	trichloroethylene
TCOH	trichloroethanol
UF	uncertainty factor